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No. 1.

ORIGINAL ARTICLES

A NEW METHOD FOR THE PRODUCTION OF GENERAL ANALGESIA AND ANÆSTHESIA WITH A DESCRIPTION OF THE APPARATUS USED¹

BY D. E. JACKSON, M.D., PH.D., ST. LOUIS, MO.

IN the following paragraphs there will be described a method for the production and maintenance of prolonged general analgesia or anæsthesia by means of nitrous oxide, ethyl chloride, ether, chloroform, ethyl bromide, "somnoform," etc., with oxygen. The method involves a continuous process of rebreathing of the gaseous or volatilized anæsthetics from which the exhaled carbon dioxide, etc., have been removed and to which oxygen is constantly added in proportions suitable to maintain the patient in a satisfactory condition. The method involves the use of special apparatus which is so arranged as to give the anæsthetist complete control of every phase of the anæsthesia at all times. In the apparatus here described great care has been taken to provide safety devices. So far I have had an opportunity to try this method only on animals, but there seems to be good reason to expect that in man results entirely comparable to those produced in animals may be readily obtainable. It is chiefly with this object in view that I have carried out a long series of experiments by this method.

A number of the basic principles upon which I have constructed the apparatus herein described have long been known to science. As early as the year 1780 Antoine-Laurent Lavoisier had isolated carbon dioxide from expired air and had shown that in respiration oxygen is consumed, thereby starting work which within the next century was to lead to a long series of brilliant investigations along the lines of general metabolism and of analysis of respired gases. Names which should perhaps here be mentioned in connection with this subject are, Magnus (1837), Bischoff (1837), Regnault and Riesel (1849), Voit (1861), Pettenkofer (1863), Andrews (1868), Pflüger (1868), Bert (1878), Zuntz (1880), C. Martin (1888), Geppert and Zuntz (1888), Luciani and Piut-

¹From the Department of Pharmacology, Washington University Medical School, St. Louis, Mo.

ti (1888), Atwater and Rosa (1899), Hewitt (1900), Haldane and Pembrey (1900), Atwater and Benedict (1905), Cushny (1909), Benedict (1909, 1912), Rolly and Rosiewicz (1911), Yandell Henderson (1910), Gatch (1911), and Meltzer and Auer (1909-11). Regnault and Riesel were probably the first to pass a current of oxygen into a small chamber in which an animal was confined for the purpose of analyzing the changes produced in the air by the respiration of the animal. They used a strong alkaline hydroxide solution to absorb the carbon dioxide output of the animal. Oxygen was added from a constant pressure reservoir. These principles have been used by practically all workers on respiratory metabolism since 1849. In the present experiments I have utilized them not for the purpose of obtaining data regarding the metabolism of the animal but with the object of maintaining conditions suitable for the normal respiration of the animal while at the same time there are added to the respiratory medium such quantities of gaseous or volatile anæsthetics as may be necessary to produce and maintain any desired and attainable degree of analgesia or anæsthesia, depending on the pharmacological properties of the substance administered. It will be noted at once that only very small quantities of the anæsthetic, such as nitrous oxide, ethyl chloride, ether, ethyl bromide, etc., are needed to produce a prolonged effect on the animal. And the depth of the anæsthesia becomes simply a matter of the degree of saturation of the animal with the anæsthetic. Since the anæsthetist has complete control of the amount of anæsthetic introduced into the animal it is obvious that when the desired degree of nervous depression has been reached no more anæsthetic need be given. The method by which these results are attained can best be explained by reference to Fig. 1 which is a diagrammatic plan of the apparatus used.

DESCRIPTION OF APPARATUS

A small electric motor (1) operates an air pump (2) which may be either of the rotary form (as shown here) or, perhaps, better of the piston form. By means of a closed system of pipes and vessels air may be kept continually circulating through the apparatus without either loss or gain of air except at the instance of the operator. Air leaving the pump at pipe (3) passes by the valve (4) unless this valve be open in which case part or all of the air will escape into the room. If the valve (4) be closed then the air proceeds through the valve (5) past the air-cock (6) and through pipe (7) into the special wash jar (9). This jar is three and one-half inches in diameter and twelve inches high and has an air-tight cover. The glass tube entering the jar is connected with the outer metal pipe by rubber hose. The glass tube makes an S-shaped bend a little distance below the cover and then passes through a bell-shaped piece of glass (8) which is firmly attached to the glass tube. Below the glass bell the tube again passes down inside a glass cylinder about two inches in diameter and six inches long. This cylinder reaches nearly to the bottom of the jar and is firmly attached to the glass tube by four glass spokes, two above and two below, each pair of spokes being placed in the same straight line, but on opposite sides of the glass tube. The glass tube lacks about one inch of reaching as low down as the outer glass cylinder. The jar is filled to the height of three or four inches from the bottom with a strong aqueous solution of sodium hydrate and calcium hydrate. The purpose of this solution is to absorb the

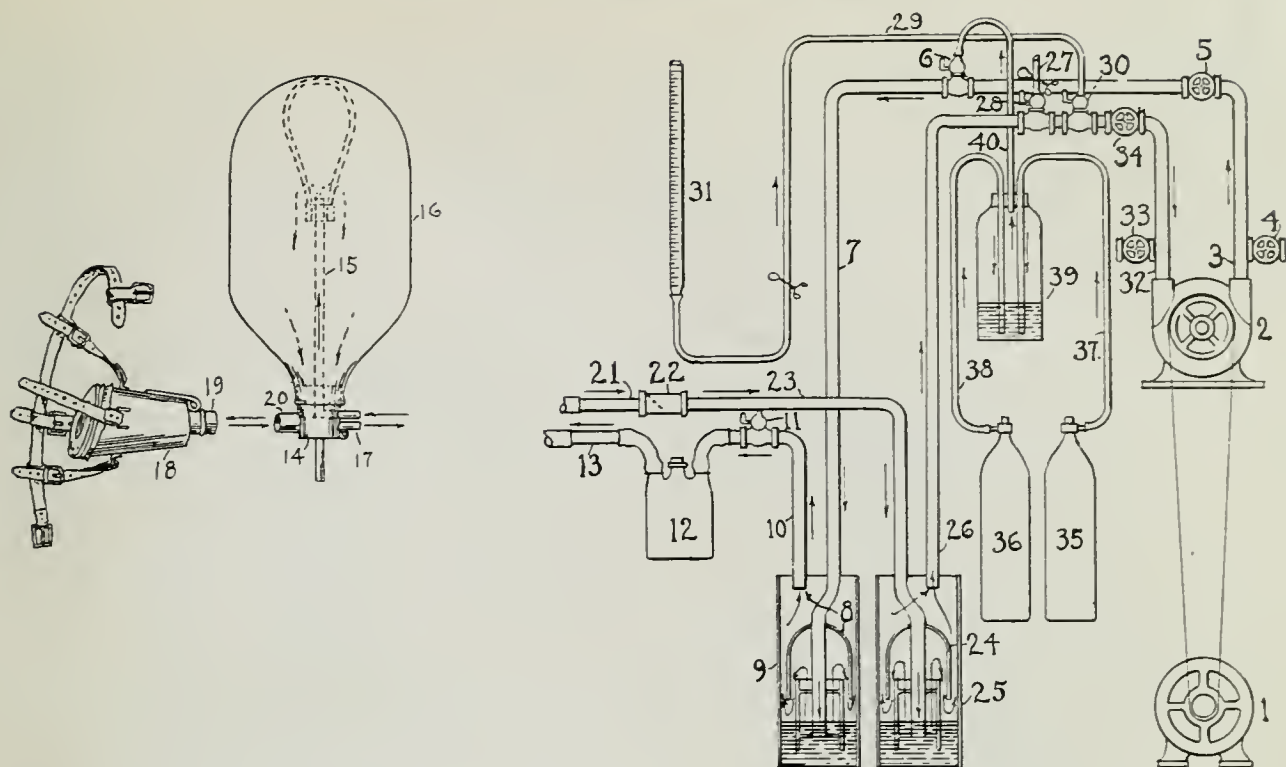


Fig. 1.—Schema showing the general plan of the apparatus together with head-piece, breathing bag and muzzle, as arranged for use with animals. The arrows indicate the direction of the air current. Number 35 represents the oxygen tank and 36 the nitrous oxide tank. In reality the apparatus carries two tanks each of oxygen and nitrous oxide. For full description see text.

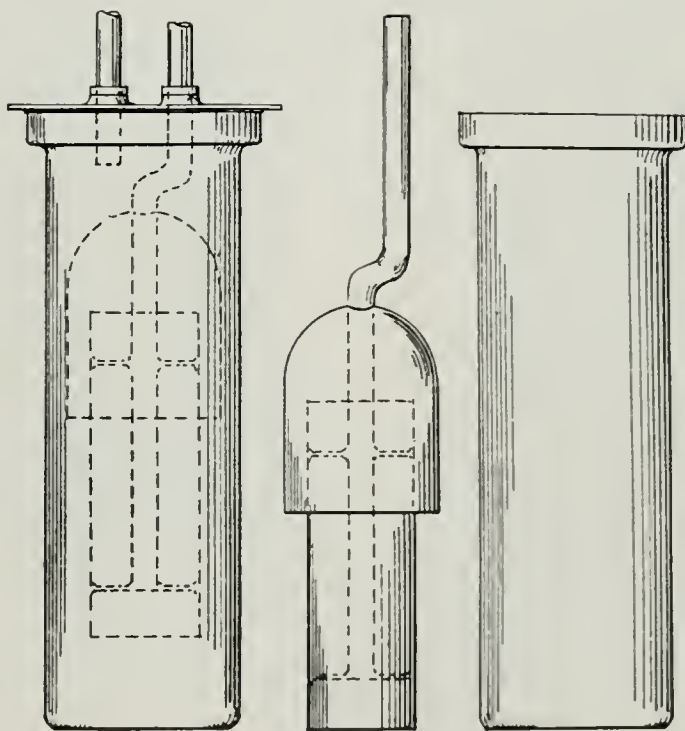


Fig. 2.—This drawing illustrates on an enlarged scale the wash jars shown at 9 and 25 in Fig. 1. For use the jars are filled with fluid to the height of 3 or 4 inches. For full description see text.

carbon dioxide produced by the animal. Air entering through the glass tube passes out of the lower end of the tube into the solution. Since there is a strong current of air circulating there is a great deal of splashing of the fluid. But this splashing is almost wholly confined within the glass cylinder. This upward splashing is entirely caught by the glass bell and directed back down into the jar. The air passes up into the bell, thence downward underneath the lower edge of the bell, thence upward into the upper part of the jar and out through

the tube (10) which leads past an air-cock (11) into a glass chamber (three-necked Woulff bottle) which serves as a safety device permitting the operator to see if any fluid, etc., is passing from the machine to the animal. In this reservoir (12) may also be placed water to moisten the air breathed (I have not found this to be necessary), sodium bicarbonate solution to further purify the air (I have not found this to be necessary), or, carbon dioxide or other drugs or gases may be introduced into it especially for experimental purposes, or, oil of bitter orange peel may be placed in it to perfume the air breathed by the patient. From this reservoir the air passes through the pipe (13) to which is connected an ordinary red antimony rubber hose about three or four feet long and having an inside diameter of three-eighths of an inch. This hose carries the air (and anæsthetic) to the face- (or head-) piece. Two special forms of face- (or head-) pieces have been made, one for dogs, the other for man. I shall confine the description mainly to the former since I wish to deal only with animal experiments in this paper.

In the animal head-piece a tapering brass cylinder about two inches in diameter at the top is closed at the bottom (14). Fastened to the bottom is a brass rod which extends downward about two and one-half inches. This rod may be inserted into a hole in the operating board and thus serve to hold the head-piece upright. Into the right-hand side of the cylinder are soldered two brass tubes three-eighths of an inch in diameter. To the outer ends of these are attached the rubber hoses passing to and from the machine. The brass tube carrying air into the cylinder turns upward at a right angle near the center of the cylinder and thus carries the air up near the top of the surrounding rubber bag. To the upper end of this tube it attached (by each end) a long coil of steel wire which forms a closed loop above the tube and serves as a flexible support for the rubber bag (16) which is slipped down over the spring. (The sides of the spring are bent in close together for removing or replacing the bag.) The lower end of the rubber bag fits snugly around the upper end of the brass cylinder. In the left side of the brass cylinder is an opening into which is soldered a brass tube (20) about one and one-eighth of an inch in diameter and about three-fourths of an inch long. The animal breathes back and forth through this tube. A large perforated cork may be placed in this tube and the side tube of a tracheal cannula slipped through the hole in the cork if one cares to open the trachea. If one does not desire to kill the animal then the metallic muzzle (18) as shown in the diagram may be placed over the animal's nose and mouth. This muzzle is a brass cylinder tapering toward the front where it ends in a large opening into which a flange (19) is soldered. This flange slips over the tube (20) on the head-piece, the joint being made air-tight by a broad rubber band. The rear end of the muzzle is slanted at an angle as shown in the diagram, and around the edges on the outside are soldered two rings of heavy wire. A sheet of heavy rubber dam is tied over the rear end, a number 18 wire being used to tie the rubber down in between the two heavy wires soldered to the rim of the muzzle. In the center of the rubber dam a round opening about one and one-eighth inches in diameter is made. This slips tightly over the animal's nose and mouth. The muzzle is held on by four straps which pass to a collar to which each is attached by buckles. I have found this muzzle fairly satisfactory. The objections are that the animal cannot open its

mouth well to breathe and saliva is liable to occasionally accumulate within the muzzle. The first objection is liable to be noticed with nitrous oxide, and the latter with irritating anæsthetics.

Within the head-piece and rubber bag a thorough ventilation of the air is assured by having the incoming air pass in near the top of the bag while

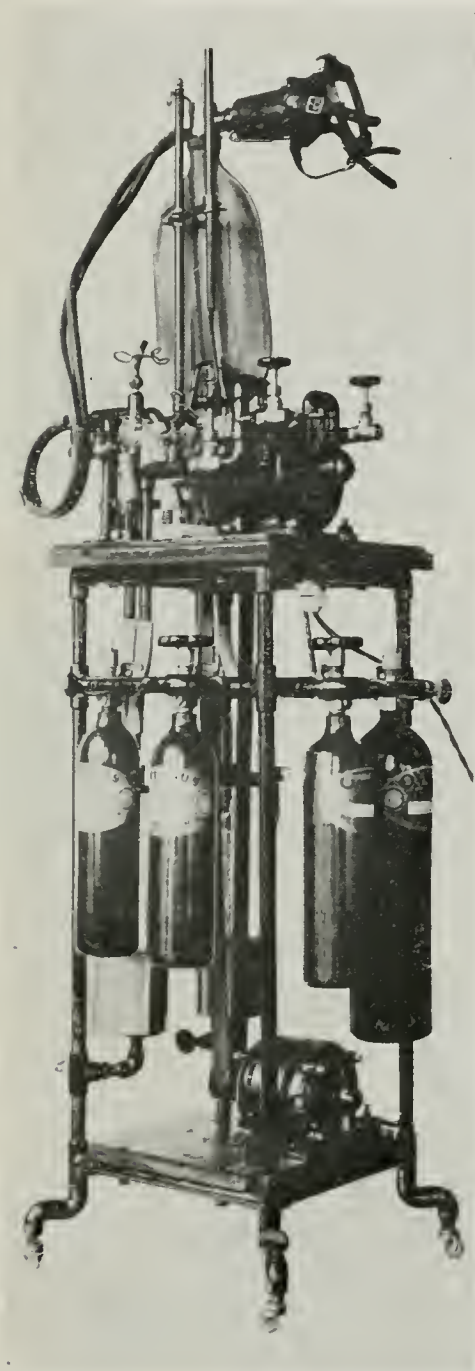


FIG. 3.



FIG. 4.

Fig. 3.—General view of the apparatus showing the left and rear sides. The muzzle, head-piece and breathing bag as arranged for dogs are shown hanging from a hook at the top of the apparatus. The rubber tubes connecting the head-piece to the apparatus are three or four feet long, thus enabling the anæsthetist to place the apparatus at a considerable distance from the animal when an anæsthetic is being given.

Fig. 4.—General view of the apparatus showing the front and left sides. The position of the wash jars is well shown.

the outlet is at the bottom. This occurs only while the pump is running. If the pump be stopped any desired amount of rebreathing may be secured. Oxygen may be allowed to reach the bag constantly or intermittently as preferred so the oxygen supply to the animal is entirely independent of the rebreathing or

of the carbon dioxide content of the bag. (These same principles apply in the face-piece which I have constructed for the human subject.) From the head-piece air passes out through the rubber hose at (17) and back to the machine with which it is connected by the metal pipe (21). These two pieces of rubber hose are clamped side by side by small flat metal clamps. They are thus

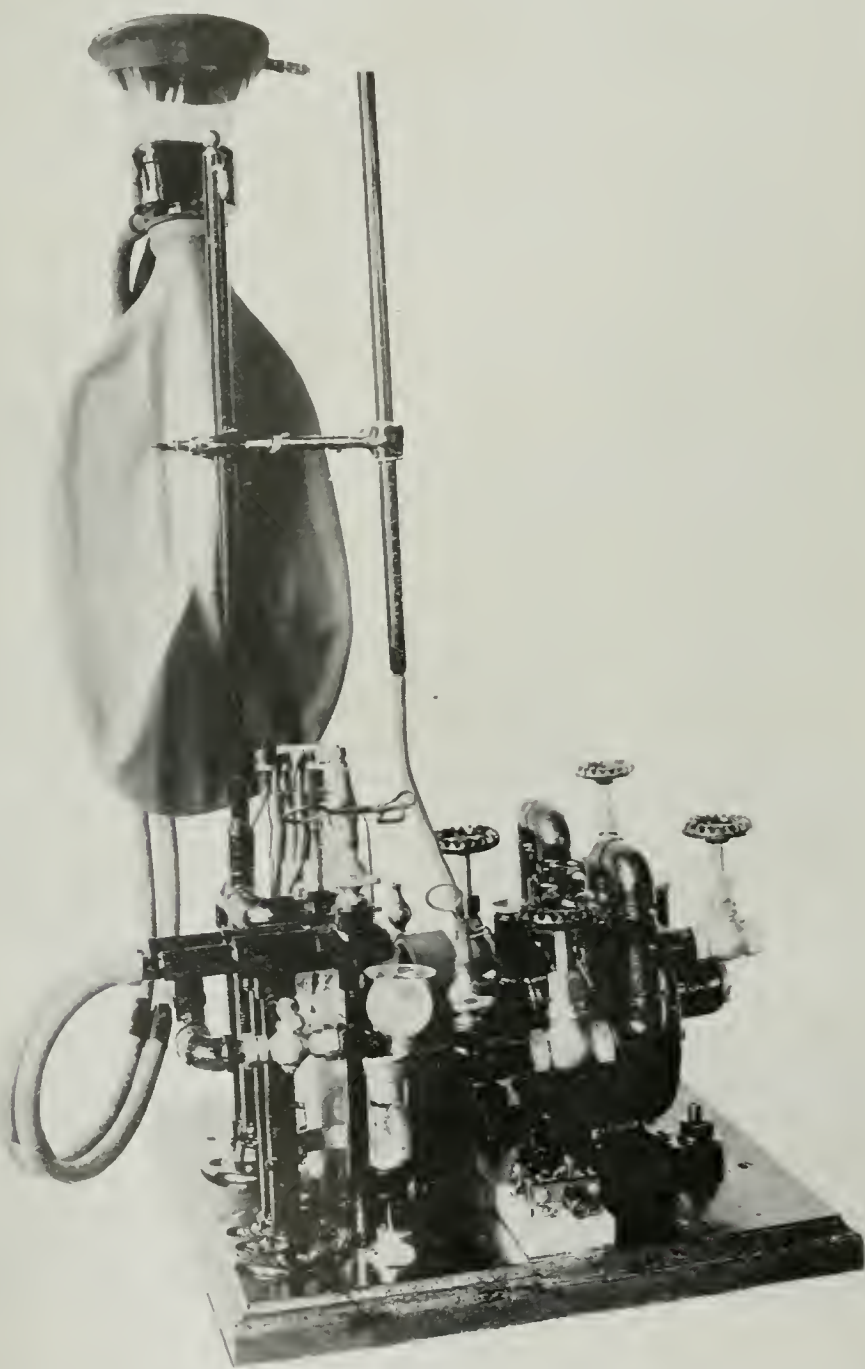


Fig. 5.—View showing the upper portion of the apparatus. The breathing bag and face-piece as arranged for man are shown hanging from a hook at the top of the apparatus.

kept almost entirely out of the way. This head-piece may also be used for intra-tracheal insufflation. The tube leading to the head-piece in this case has a Y-tube inserted in its course near the brass cylinder of the head-piece. From the two forks of the Y-tube short rubber tubes carrying screw clamps pass to the cylinder. One of these tubes connects onto the inlet tube as usual. The

other rubber tube is connected with the inside of the cylinder by means of a small metal or glass tube passing through a cork inserted in an opening in the brass cylinder (not shown in the diagram). From this small tube inside the cylinder a catheter may be passed out through the flange (20) and either enter the trachea directly or pass in at the side tube of a tracheal cannula. The correct amount of pressure for the intra-tracheal tube can be obtained by adjusting the screw clamps on the short rubber tubes coming from the Y-tube. (I believe this same principle may very well be used in man, possibly even for nitrous oxide anæsthesia).

Air passing from the animal back to the machine enters the pipe (21) and soon reaches a check valve (22) which is intended as a safety device to pre-

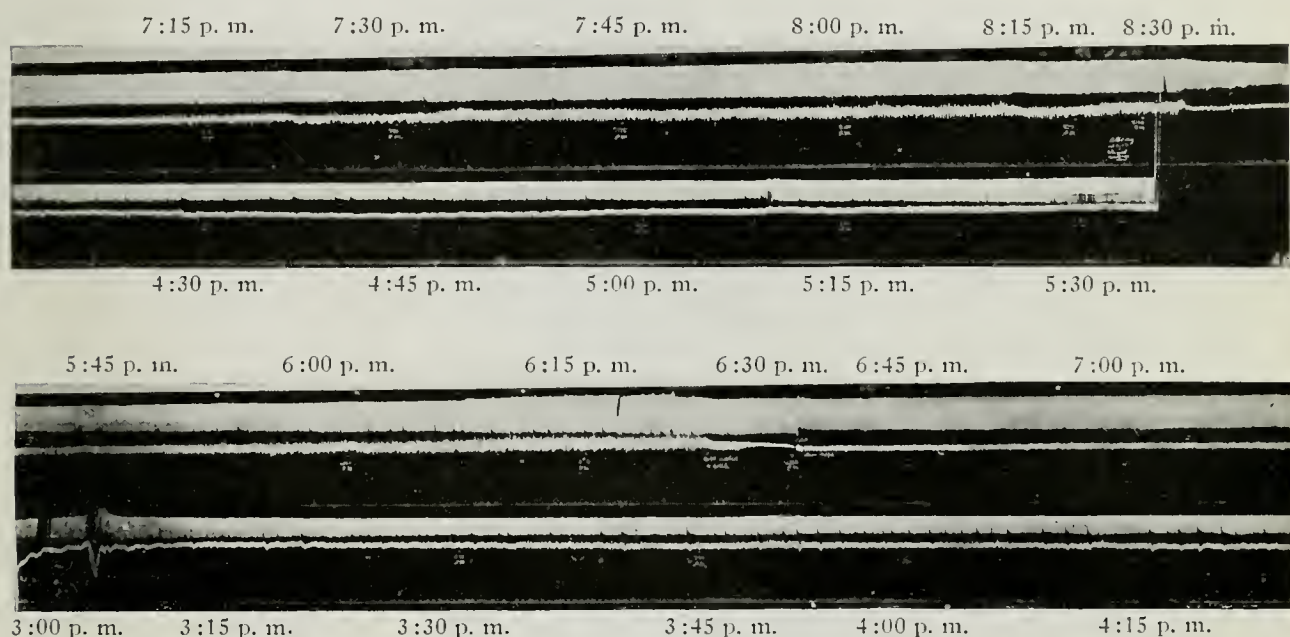


Fig. 6.—This is a photographic reproduction (in two sections) of the blood pressure and respiratory tracings from a dog under *nitrous oxide* anæsthesia. The original tracing formed a single record eighteen feet long as recorded on a slowly moving drum. The total length of duration of the anæsthesia was a little more than *five and one-half hours* and the animal was in excellent condition at the close of the experiment. The time is marked on these records in fifteen-minute intervals. A few breaks in the course of the tracings are due to clots in the arterial cannula, readjustment of the apparatus, etc. The signal magnet (at the base line) shows five-second intervals.

vent any reversal of the air current. From this valve the air passes through the pipe (23) to a second wash jar (25) exactly like the first but containing concentrated sulphuric acid which removes excess moisture from the air. This acid also serves the further purpose of being a good sterilizer of the air leaving the animal's lungs. And the faint possibility of any organic poison being exhaled by the animal is also provided against by the acid. In addition, by placing the acid here, the air which passes on to the pump is made relatively dry and this serves to prevent the pump from rusting.

From the wash jar (25) the air passes through the pipe (26) back toward the pump. But at a short distance from the pump two air-cocks are connected with the tube. Through the first of these cocks (28) such volatile substances as ethyl chloride (kelene), ethyl bromide, somnoform, etc., may be sprayed into the machine through a short piece of rubber tubing carrying a clip (27). Still nearer the pump is another air-cock (30) through which less volatile substances such as ether or chloroform (or ethyl bromide) may be injected from a burette (31). This permits of very accurate dosage, but in this connection

one must remember that it is necessary to saturate the air and solutions in the machine to a certain definite degree in order to secure and maintain any definite degree of anæsthesia in the animal. This can be done, however, approximately at least, by injecting some ether, etc., into the machine and running the

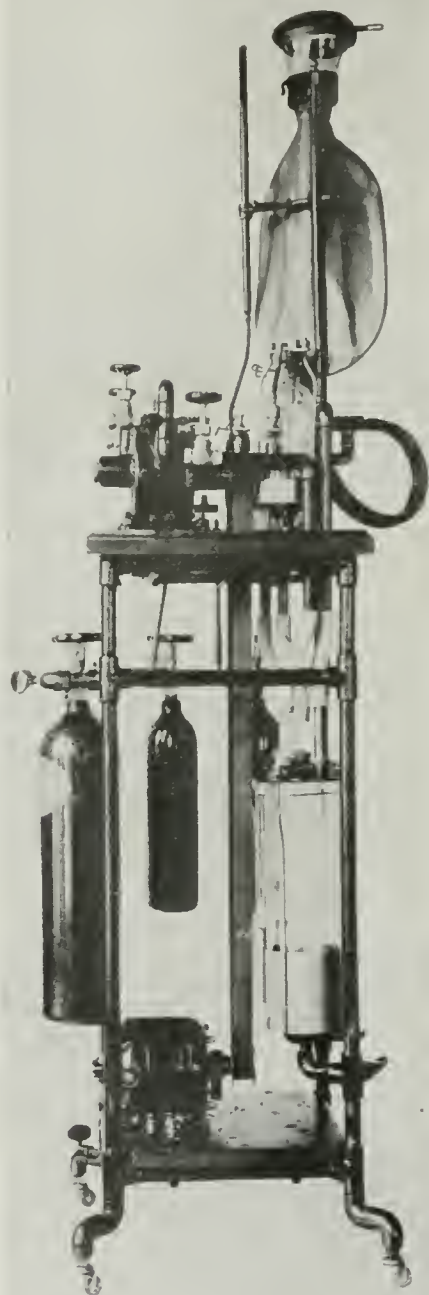


Fig. 7.—General view of the right-hand side of the apparatus. The face-piece and breathing bag as arranged for man are shown at the top of the apparatus. Just to the left of the bag is shown a twenty-five cubic centimetre burette (graduated to tenths of a c.c.) used for the injection of ether, chloroform, etc., into the air system.

pump for a little time before the machine is connected to the animal. It is advisable to do this also with nitrous oxide.

Still nearer to the pump is another valve (34) which is placed in the main air circuit. This valve corresponds with the valve (5) which is also placed in the main air circuit. Valve (33) opens to the outside air. If, while the pump is running, valve (4) be slightly opened a little air will be forced out of the system into the room. This will instantly reduce the quantity of air in the re-

spiratory bag. By watching the bag as one opens the valve (4) cautiously the tension of the air in the bag can be immediately reduced to any desired degree. Conversely by slightly opening valve (33) any desired quantity of air can be drawn into the system. These two valves give the operator complete control at all times of the amount of air in the machine and these adjustments can be made in a few seconds. Through pipe (32) the air current again enters the pump and passes out through pipe (3). This completes the circuit of the air which has lost some of its oxygen to the animal but has gained some carbon dioxide and watery vapor. The carbon dioxide in the next few rounds will be taken up by the soda-lime while the watery vapor will be taken up by the sulphuric acid. The oxygen, however, must be replaced from the outside. This is accomplished by injecting oxygen from an ordinary commercial oxygen tank (35) into the air circuit. From the tank a tube (37) leads to a wash bottle (39) containing water or sodium bicarbonate solution through which the oxygen passes. From the top of this wash bottle a tube leads to the air-cock (6) through which the oxygen is passed into the air system. The extent of distension of the bag and the symptoms of the animal serve as guides for the administration of oxygen.

Nitrous oxide may also be administered in the same manner as the oxygen as shown in the diagram. Carbon dioxide may also be injected into the system from an ordinary commercial tank of the gas by way of the air-cock (11) just in front of the reservoir (12). Number 36 represents a tank of N_2O .

THEORETICAL DISCUSSION

It is obvious at a glance that no anæsthetic can be used in this machine which will undergo decomposition or be chemically changed by contact with concentrated sulphuric acid or sodium or calcium hydrate solution. By a peculiarly fortunate coincidence sulphuric acid and sodium hydrate solution are the two most common reagents used in the purification of a majority of the anæsthetics in general use. And in this machine the anæsthetic undergoes a constant purification. I have suspected this might be of some real benefit practically. So far I have successfully used nitrous oxide, ether, chloroform, ethyl chloride, ethyl bromide,² and the proprietary preparation "somnoform." While my original object was mainly the production of a method by which nitrous oxide anæsthesia might be made cheaper and safer, thereby extending its use particularly to that large and unfortunate proportion of our population which must depend on charity for its surgery, I am now inclined to believe that the method may well be used for a majority of the anæsthetic substances now known.

It is one of the basic principles of pharmacology that most anæsthetic substances which are administered through the lungs by inhalation are almost totally excreted again by exhalation from the lungs. Such small quantities of the common anæsthetics as might disappear in the tissues would probably have no influence whatever on the use of this method. By means of the constant circulation of the air within the machine the exhaled anæsthetic is simply carried around, washed through the sulphuric acid and sodium hydrate solution and again returned to the animal for rebreathing. It is thus seen that so long

²Ethyl bromide "is with much difficulty saponified by potassium hydroxide and it is not attacked by sulphuric or nitric acids" (New and Nonofficial Remedies, 1915, p. 26). I have not experienced any difficulty in this direction in the use of ethyl bromide.

as no air is allowed to enter the machine from without and none is allowed to escape from within, the concentration of any given anæsthetic vapor within the machine and in the tissues of the animal must remain practically constant. Consequently the degree of anæsthesia should also remain constant. It is easy enough to carry this out with such a substance as ether. But it is somewhat more difficult with the milder anæsthetics such as nitrous oxide or ethyl chloride. With these bodies it is often difficult for one to interpret the degree of analgesia or anæsthesia present in dogs. I suspect it might be easier to do this in man. In dogs under ethyl bromide it may sometimes be found, for example, that touching the cornea will not cause winking and yet the animal may struggle about rather violently entirely independently of the carbon dioxide or oxygen present, and when one may feel very sure that the animal is entirely unconscious of painful sensations. One is always tempted then to give more of the anæsthetic, but a slight increase in the concentration of the vapor may throw the animal into a dangerous condition. It would seem probable in such cases that the drug may have been performing its full therapeutic function as an analgesic and if one could control the struggling this analgesia might easily be maintained for a prolonged period. I suspect that, particularly with ethyl chloride, this might be done in the human subject (e. g., in obstetrics) very much more satisfactorily than with the dogs on which I have experimented. With ether, however, one can check the struggling at an early stage of the anæsthesia and by carefully injecting small quantities ($\frac{1}{2}$ to 1 c.c.) at a time the animal may be brought to a condition in which it will just wink the eye when the cornea is touched and yet the skeletal muscles will all be relaxed and no voluntary movements may occur. One may stop giving the anæsthetic at this point and then maintain this stage of anæsthesia for twenty or thirty minutes. But if the degree of saturation of the tissues of an animal with an anæsthetic remains constant then the animal will tend to gradually fall more and more under the influence of the drug.

It is neither necessary nor desirable that the pump be running continuously. It has been repeatedly and most excellently shown by Yandell Henderson that a moderate accumulation of carbon dioxide may be of decided benefit to an animal under anæsthesia. By simply stopping the pump in this machine one can allow any extent of rebreathing or of carbon dioxide accumulation he may desire. And during this period he may or may not give the animal oxygen as he sees fit. Or he may even give the animal carbon dioxide from a tank if he wishes to do so. On the other hand if one prefers he may keep the pump running all the time and thereby almost entirely prevent any carbon dioxide accumulation within the breathing bag.

It has been repeatedly shown that warmed vapors are more satisfactory for anæsthesia than those which are cold. This machine automatically warms all gases or vapors circulating through it to approximately room temperature. This is accomplished first by the sulphuric acid which becomes warm from the absorption of moisture and second by the pump which develops heat from its friction in turning and by compression of the air passing through it. The alkali solution usually remains at practically the same temperature as the room and this determines the temperature of the air as it passes into the bag. The bag should be large, perhaps about one and one-half or two times the vital

capacity of the animal (or even larger). I have tried several different bags. In every case I have found that the larger the bag is the better the results are. When the air reaches the bag, which is made of thin rubber and has a large surface, then the temperature of the air in the bag should soon reach almost exactly that of the surrounding atmosphere. In rebreathing the animal itself quickly warms the air in the bag.

The air breathed by the animal should be properly moistened (perhaps of between 50 and 70 per cent relative humidity). I have had no difficulty in this respect with the machine. But if desirable water may be placed in the reservoir (12) to moisten the air before it passes to the bag. Too much moisture in the air I believe can be satisfactorily provided against by increasing the quantity of sulphuric acid in the wash jar [or possibly by placing acid in the reservoir (12)].

I have, unfortunately, not been able so far to make any very careful *practical* determination of how much sodium (and calcium) hydrate may be required for this method of anæsthesia by the hour. The amount must necessarily vary greatly in different animals and with different anæsthetics. The cost of the oxygen will probably represent the chief expense of anæsthesia by this method. And this is determined by the amount of the gas *actually consumed* by the animal. The sulphuric acid may be purchased very cheaply and this expense cannot be great. It should be possible to calculate approximately the amount of hydroxide and of oxygen which would be required for an anæsthesia of any given length of duration. "In an average man weighing 70 kilos the mean production of carbon dioxide is about 800 grammes (400 litres) in twenty-four hours, and the mean consumption of oxygen about 700 grammes (490 litres).³ But there are very great variations depending upon the state of the body as regards rest or muscular activity, and on other circumstances. In hard work the production of carbon dioxide was found to rise to nearly 1,300 grammes, and in rest to sink to less than 700 grammes, the consumption of oxygen in the same circumstances increasing to nearly 1,100 grammes and diminishing to 600 grammes. In rest, in moderate exertion, and in hard work, the production of carbon dioxide was found to be nearly proportionate to the numbers 2, 3, and 6 respectively."⁴

The production of carbon dioxide in an animal under deep anæsthesia must be very much less than during conditions of normal activity. And a corresponding decrease in the consumption of oxygen also occurs under the anæsthetics. It is often striking to observe during an anæsthesia by this method how small a quantity of oxygen is really required in order to keep the circulation and respiration in good condition. Using the above data as given by Stewart I have made a rather unsatisfactory theoretical calculation which shows that the cost of nitrous oxide anæsthesia for a dog weighing 15 lbs. (1/10 the weight of an adult man) should be approximately \$0.0399 per hour. I believe that I have used more material than this figure would indicate in my experimental observations, especially of oxygen and sodium hydrate. The nitrous

³It will require 1,455 grammes (3.2 lbs.) of pure NaOH to change 800 grammes of CO₂ into Na₂CO₃. This hydrate would cost about \$1.28. Commercial NaOH is seldom pure, however. Seven hundred grammes of oxygen per 24 hours corresponds to about 5.36 gallons per hour. In large cylinders oxygen sells for about 2 cents per gallon, in small cylinders at from 4 to 6 cents per gallon.

⁴G. N. Stewart, *Manual of Physiology*, 5th edition, p. 214.

oxide which I have used is, I suspect, within the limits of its relative proportion of the cost indicated. I have, however, wasted a good deal of oxygen and sodium hydrate in various experimental procedures. The cost should be less with ether or chloroform than with nitrous oxide. With respect to ethyl chloride, ethyl bromide or "somnoform" I have usually found from five to ten cubic centimetres sufficient for a fifteen pound dog. (From ten to twenty cubic centimetres would probably be sufficient for a man for the "dead space" of the machine requires as much vapor for a dog as for a man.) These drugs are best used when supplied in small glass ampouls of 3 c.c. or 5 c.c. capacity. A short rubber tube with a piece of cotton or gauze in the lower end is attached to the nozzle of the air-cock (28) and the drawn-out end of the ampoul is inserted like a cork (air-tight) into the upper end of the tube. The cock is then opened and the pump started. When all is ready the end of the glass ampoul is snapped off by bending the rubber tube and the drug quickly enters the air system. One must watch that the sudden entrance of a large volume of vapor does not over-distend the breathing bag. This can usually be checked by stopping the pump suddenly which tends to retard the passage of the vapor forward into the bag. For this reason small ampouls of very volatile bodies are better than large ones.

The duration of anæsthesia by this method is usually limited only by the convenience or desire of the operator. With nitrous oxide I have been easily able to keep dogs anæsthetized for periods of time extending up to a little more than *five and one-half hours* and the animals are in excellent condition at the close of the anæsthesia. The animal may often stand up and walk around the room within one and one-half or two minutes after it is removed from the nitrous oxide, but after a very long anæsthesia usually from five to nine minutes are required for complete recovery. With ethyl chloride one and one-half hours is the longest time that I have so far tried to keep an animal anæsthetized. (This is not a good anæsthetic for dogs.) With ethyl bromide I have kept dogs anæsthetized for periods up to a little more than an hour. With ether or chloroform the duration of the anæsthesia may be regulated practically entirely by the desire of the anæsthetist.

SUMMARY

1. A new method for the production and maintenance of general analgesia or anæsthesia is described.
2. A description of the device required for the production of analgesia or anæsthesia by this method is given.
3. Great care has been exercised to confine the descriptions in this article strictly to results which have already been obtained by experiments on dogs. (But the conclusion is easily drawn that similar results may be obtained in man.)

SPECIFIC TREATMENT IN TYPHOID FEVER

BY FREDERICK P. GAY, M.D., BERKELEY, CALIF.

THE history of the specific treatment of typhoid fever divides itself readily into two definite eras, the first beginning with the work of Fraenkel¹ in 1893 and extending approximately for 20 years to 1912, and the second from the latter date to the present time. In offering this critical summary of the past history, present status, and probable future of a specific therapy against this malady, we may, to a large extent, omit a detailed consideration of the facts of the first of these two eras that has been mentioned, since it has been fully reviewed repeatedly in the past by each successive observer of the methods concerned. A rather full discussion of this subject will be found in the articles by Callison² and by Krumbhaar and Richardson,³ and particularly in the systematic treatises on Typhoid Immunization by Fornet⁴ and Friedberger.⁵

As has been already stated, Fraenkel in 1893 reported the results he obtained in treating 57 cases of typhoid with cultures of *B. Typhosus* that had been heated to 60 degrees. The vaccines were administered hypodermatically and fairly good results were obtained. The specificity of the action of typhoid vaccine that was assumed by Fraenkel in those of his cases which did well, was subsequently open to doubt from observations of Rumpf⁶ who claimed that he obtained equally good results by using cultures of *B. Pyocyaneus*. The accuracy of his observations, however, was subsequently questioned by Kraus and Buswell⁷ and Presser.⁸ Petruschy⁹ in 1902, used typhoid vaccines in combination with immune serum and in several cases found this treatment was followed by a fall in temperature during the subsequent three or four days. Pescarolo and Quadroni¹⁰ later used living avirulent cultures in treatment.

By far the greater number of reports on the use of vaccines in the treatment of typhoid fever during this earlier period were made by English and American writers, and did not differ essentially in the results obtained or in the methods employed. Ordinarily heat-killed vaccines (56 degrees—60 degrees) were employed in variable dosage and at varying intervals during the course of the disease. Fornet⁴ has summarized the results of 15 authors who report more or less favorably on the results obtained in over 300 cases. Callison² has extended this list to include some 747 cases. The consensus of opinion, with few exceptions, of the authors during this period, is that the use of the vaccines when given under the most favorable conditions of dosage and interval, shortens the course of the disease, lessens mortality, diminishes the percentage of relapses and complications, and may, in some instances, give rise to rapid defervescence and cure. We are, in these cases, at best dealing with relatively small differences in percentage in comparing the treated cases with those that have been used as controls. The instances of abortion of the disease, although exceptional in this series of cases, was mentioned as early as the work of Fraenkel who obtained it in two or three cases. A further consideration of this rapid amelioration, which apparently followed the use of vaccines, will be considered in more detail in connection with the more recent

methods of treatment. At best, no very striking results can be claimed for the use of the ordinary vaccines administered subcutaneously in the treatment of typhoid fever.

In addition to the use of the more usual types of vaccines, it may be mentioned that autogenous vaccines have been suggested by various writers, notably Thierloix, Garsaux and Bardon;¹¹ Josue and Belloir;¹² Sacquepee and Chevrel¹³ and Bourke, Evans, and Rowland.¹⁴ In a similar way Ramond and Goubert¹⁵ have suggested a process of autohemotherapy by subcutaneous injections of the patient's own blood drawn from the veins and injected directly into the adjacent connective tissue. By such means they claim a distinct benefit in 38 per cent of their cases. Bourke, Evans and Rowland have suggested using 60 to 300 millions of living typhoid bacilli derived from the blood of the patient who is treated.

Before passing to the more recent work in the use of vaccines, reference should be made to attempts at the treatment of typhoid fever by means of antiserum or antitoxins. The most favorable results that have been reported by this method are those of Chantemesse¹⁶ who produced typhoid vaccines by growing the bacteria on extract of spleen and immunizing horses with this preparation and later with the living cultures. By means of serum obtained in this manner Chantemesse claims to have reduced the mortality in his wards from 17 to 4.3 per cent. Rodet and Lagriffoul¹⁷ have reported the use of an anti-typhoid serum prepared by themselves, in 65 cases, which seems to shorten the course of the disease and in some cases to abort it if given early, that is before the tenth day. Ludke¹⁸ immunized goats against toxins and living cultures of typhoid and claimed favorable results on patients given intravenous injections of this immune serum. Less favorable results have been reported in the use of antiserum prepared by Besredka, by Andriescu and Ciuca,¹⁹ and by Ciuca, Combiescu and Balleanu.²⁰ Moderately good results have been reported by Gaupp²¹ who used an antiserum prepared by Kraus in 16 cases. Koenigsfeld²² injected the serum of the individual patient in doses of 2 to 4 c.c. after the Widal had become well established, and found a distinct, though slow, improvement of the disease in 14 out of 18 cases. Ramond and Goubert¹⁵ have reported good results in 38 per cent of cases by means of an autohemotherapy. Perhaps the best comment on the use of antiserum in typhoid fever is the short list of those who have described its effects at all, and the fact that many of those authors have subsequently reverted to the use of vaccines.

Interest in the specific treatment of typhoid fever has been rapidly developing during the last three years, and particularly during the last year and a half. Not only has the number of publications been larger than during the twenty preceding years of attempt at specific therapy, but the results obtained are far more striking and of a distinctly more convincing type. As in the previous years the best results have been obtained by the use of vaccines, but two important modifications in the vaccine treatment of typhoid have recently been introduced; namely the use of sensitized (Besredka) vaccines instead of ordinary vaccines; and second, the intravenous injections of the vaccines themselves. These two modifications, either employed separately or more particularly when combined, had led to a surprising increase in the percentage of aborted cases of the disease, the infrequent occurrence of which has already been noted as

having been observed from the time of Fraenkel. Not only does the very considerable per cent of cases that may be aborted by the more recent methods lead one to feel confident that methods have finally been determined, which, from the results obtained, are distinctly indicated as a rational treatment, but the results themselves give far greater confidence that they are due to the treatment and not to chance, spontaneous cures. It is of course recognized that rarely in cases of typhoid fever, during the height of the fever and perhaps even early in its course, the temperature may fall by crisis or lysis. When such results, however, are obtained in 30 to 50 per cent of the cases, and early in the disease, the element of spontaneous cure may be safely eliminated when the results follow directly after specific treatment.

Kraus and Mazza²³ and Kraus²⁴ reported in 1914, the favorable results that, in a number of cases, followed the intravenous injection of 50 to 100 millions of Vincent's typhoid vaccine (polyvalent and ether killed). Such injections are followed by a rise in temperature of 1 to 2 degrees, with a chill and then a rapid fall in temperature to normal or subnormal which may remain permanent. Thioloix and Bardon²⁵ reported that they also obtained better results when typhoid vaccines are given intravenously and when they are autogenous. The fact that this action is not strictly specific has been evidenced by the work of Kraus²⁴ who found that he produced similar results in typhoid fever by the use of *B. coli* vaccine. Lüdke²⁶ claims to have actually cured certain cases by the use of deutero-albumose. Rhein²⁷ used the Halle vaccines diluted to a considerable extent and also administered intravenously. In a series of 33 cases he found that abortive cure took place within two days in 30 per cent, and in 48 per cent the fever was distinctly shortened.

But even better results have been obtained by the use of sensitized typhoid vaccines. These vaccines, which were originally suggested by Besredka in 1902, consist in either living or dead bacteria that have been treated, or sensitized, by means of an immune serum which is active against the organism in question, with subsequent removal of the excess of serum by washing. Such vaccines, particularly in the case of typhoid fever, have been shown, on the basis of the experimental work of numerous observers, to possess the following advantages over the ordinary, untreated vaccines. They give rise to transitory, passive immunity followed by active immunity that is as lasting as that secured by using untreated vaccines. They have the further advantage of producing distinctly less symptomatic disturbances than the ordinary vaccines. In their experiments on apes Metchnikoff and Besredka²⁸ found distinctly better protection against typhoid fever, which can be produced in these animals, by vaccinating with living, sensitized typhoid bacilli than by some of the other vaccines that have been devised, and used for the production of immunity in human beings. These sensitized vaccines have subsequently been extensively used for prophylactic immunization with success. In recent publications Gay and Claypole²⁹ have shown that either living or dead sensitized typhoid vaccines, but particularly the sediment of the alcohol-killed sensitized vaccines, protect rabbits against becoming typhoid carriers better than several other unsensitized preparations that were tried. For a further discussion of the use of these vaccines in prophylaxis, we refer the reader to this article.

Boinet,³⁰ in 1913, reported the use of Besredka's living, sensitized vaccines

in 15 cases of typhoid fever. When administered subcutaneously these vaccines were found to shorten the course of the disease distinctly and without any harmful effects. Ardin-Delteil, L. Negre and Maurice Raynaud³¹ obtained similar results. The same authors further showed that although animals and patients treated by sensitized vaccines did not produce high agglutinins, the bacterial substances of their blood are distinctly increased, which results correspond exactly to those of Garbat and Meyer,³² in experimental animals. Rouques³³ found that relatively large doses of Besredka's vaccine apparently led to distinct symptomatic improvement in a number of cases he observed. This was particularly true when the vaccine was used early in the disease.

Ichikawa³⁴ was the first to suggest the use of sensitized vaccines intravenously. He treated typhoid bacilli with the serum of patients who had recovered from the disease, injected this sensitized vaccine intravenously and obtained a considerable number of abortive cures of the disease by such means. He was able, moreover, to obtain almost equally good results in the treatment of paratyphoid fever with these same sensitized typhoid vaccines, indicating again, as did the work of Kraus, that the results obtained are not strictly specific. Garbat³⁵ who used a method essentially similar to that of Ichikawa, except that the vaccines were given subcutaneously, found favorable results in 17 cases, but only two abortive cures. His communication illustrates the superiority of the results attained by intravenous injection as introduced by the Japanese investigator. Paltauf³⁶ has reported similar results obtained by Biedl and by Eggerth, who compared the use of Besredka's living sensitized vaccines, intravenously, with Vincent's killed polyvalent, unsensitized vaccines likewise delivered directly into the veins. Far better results were obtained with the sensitized vaccines. Whereas Biedl reports only moderately good results, Eggarth in his letter to Paltauf, and also in a subsequent communication³⁷ succeeded in aborting the disease with a single injection in 31 out of 43 cases (72 per cent). Feistmantel³⁸ likewise found Besredka's vaccine to give better results on intravenous administration than the untreated vaccines of Kolie. He gives the treatment on four successive days in relatively small doses, and in 52 cases produced a rapid cure with critical and rapid lytic fall of temperature. These observers all agree that the method is harmless. The only dissenting report is from Sladek and Kotlowski³⁹ who, on the basis of four cases, in which distinctly good results were produced, emphasize the possible danger of collapse and heart failure.

Reference has already been made to the work of the author and Claypole²⁹ which led us, from experiments on rabbits, to believe that the best typhoid vaccine for prophylactic purposes consists in the sediment of alcohol-killed, sensitized bacteria from which the endotoxins have been removed. Good protective results with little symptomatic disturbance at the time of the injection, have been obtained with this vaccine in human beings. Certain of our experiments with a phenomena described as specific hyperleucocytosis⁴⁰ led us to suggest the intravenous injection of this sensitized vaccine in the treatment of typhoid fever. We found that the injection of typhoid bacilli in specifically immunized rabbits gave rise to an acute hyperleucocytic crisis in about 18 hours which frequently reached an extraordinary height (150,000 or more leucocytes to a cubic millimeter) and which was coincident with the sterilizing of the body from

the injected living bacteria. It seemed evident that this high leucocyte count was brought about by the increased attractiveness of the injected bacteria when they had been acted upon by the immune serum in the animal's body, for leucocytes, since it was found that a similar specific phenomena could be produced by injecting sensitized typhoid bacilli, that is bacilli treated with immune serum, intravenously in normal rabbits.

These results and the apparent relation of this increase in leucocytes to the recovery of the animal from the infection, led us to hope for further results in treating cases of typhoid fever intravenously with sensitized vaccines than with the unsensitized vaccines. Although this means of treatment was in mind before the publications on the intravenous injections of sensitized culture that have been mentioned, no opportunity was available for trying out this method until recently. Owing to the courtesy of a number of physicians in Oakland, Berkeley and San Francisco, we have recently been able to treat a few cases of typhoid fever and have obtained favorable results in harmony with those writers that have been mentioned, in a distinctive percentage of cases, and also certain indications which may lead to a further perfecting of the method. We have given intravenous injections of our sensitized vaccine sediment in 19 cases of typhoid fever, in which the diagnosis was fully confirmed by laboratory as well as clinical means. In 14 of these only was it possible to carry out the treatment exactly as intended, and these cases only are used as a basis of study. In this preliminary report we prefer to deal only with those cases in which an abortive cure was effected, following one, or in some instances, more injections of the vaccine. We have left out of consideration those cases in which just claim to shortening the duration of the disease or amelioration of symptoms could properly be made, owing to the impossibility of controlling such apparent improvements in a small group of cases. Of the 14 cases considered, 5 were cured abortively, or 35 per cent. Of these cases, two received a single intravenous injection; two received 2 injections and one received 3 injections before the temperature came to normal permanently. The average day on which the temperature reached a permanent normal in these cured cases, was the 19th. We find that adult patients readily tolerate the intravenous injection of a suspension containing about one-twenty-fifth milligram of sensitized vaccine sediment, which corresponds to 300 million of the living organisms. This dose has, in no instance, produced severe symptoms and may readily be increased on subsequent injections, when such prove necessary. The only alarming symptoms that have been noted were in connection with a patient who received by error an injection of 800 million bacteria and in this case there were slight hemorrhages from the mouth and distinct evidences of collapse which, however, were only temporary. The symptoms produced by the intravenous injections of vaccine in the cases mentioned, agree entirely with those that have been noted by other observers, notably by Ditthorn and Schultz;¹¹ Kraus and Mazza;²³ and Kraus.²⁴ In from 15 to 30 minutes following the injection the patients experience a distinct chill which may be decidedly unpleasant. The temperature rises from 1 to 2 degrees and we find that coincidentally there is a distinct fall in the number of polymorphonuclear-leucocytes, say from 6,000 or 7,000, to 3,000 or 4,000. In one or two hours the temperature begins to fall, the patient often perspires freely and experiences a very marked amelioration of general symp-

toms. At about 12 hours after the injection the temperature has reached normal or subnormal, and the leucocytes in most cases have increased, in some cases to as high as 20,000 to 40,000 per cubic millimeter. We have not as yet sufficient data to state whether the degree of hyperleucocytic crisis is of prognostic significance.

In our series of cases one relapse and no complications have occurred, so we are unable to state whether the treatment has affected them or not. Although the earlier writers on the use of typhoid vaccine seemed to think that subcutaneous doses on several occasions had a beneficial effect in diminishing the number of complications and relapses, later writers, Guinon and Malarte;⁴⁰ Ditthorn and Schultz;⁴¹ Goldscheider and Aust;⁴² and Csernel and Marton⁴³ find complications unaffected by vaccine particularly in those cases where if favorable results occur they are of the abrupt, abortive type. We have had two fatal cases, one of which died early in the disease, following several days of coma and delirium, and the other distinctly later, of no recognized cause, the autopsy being refused. Neither of these cases was apparently affected favorably or unfavorably by the treatment, with the exception that the temperature fell following nearly every dose that was given, and there was a temporary amelioration of symptoms.

We seem to have the best results by repeating the injections, when necessary three or four days apart. This allows for an abortive cure following the first injection, for although the temperature falls to normal the first day it may fluctuate for a day or two subsequently rising to 100 or 101 degrees and at the end of this time fall to normal and remain there permanently. In our experience if the second or third injection fails to produce more than temporary effect, little can be expected from further injections in the way of abortive cure. The procedure of other investigators may be mentioned in this connection simply as showing the variation in methods of dosage and intervals than have been employed. Kahn⁴⁵ advocates the use of 3 million to 5 million organisms given subcutaneously at intervals of three or four days. His favorable results, when present, are not, however, of the abortive type. Pensuti⁴⁶ gave 400 million gradually decreasing on successive days to 100 million subcutaneously and obtained a percentage of abortive cures. Kraus²⁴ gave 50 to 100 million of Vincent's vaccine, intravenously. Goldscheider and Aust⁴³ have used 250 to 750 million of unsensitized vaccine subcutaneously. These variations in dosage mean little beyond the limited experience of the author, and depend on the type of vaccine and the results desired. In our experience when an abortive cure is attempted enough vaccine must be given to produce a distinct reaction in the way of a chill with rise and fall of temperature. When treatment is directed toward symptomatic improvement, the dosage of course, as with the older types of typhoid vaccines, may be smaller.

It is of particular interest to us to note that there has been apparently some relation between the absence and occurrence, and indeed of degree of occurrence, of the Widal reaction and the results obtained. All of the cases that have been cured abortively by intravenous injections of vaccines have had a positive Widal, and in several instances a Widal that was distinctly high, for example 1-320 or 1-640. In several cases the exact titer of the agglutinin was not obtained, and in some cases may have been higher than the simple diag-

nosis of "positive" would indicate. In two at least of our failures the Widal has been persistently negative when treatment was attempted, and in none of them did the agglutinin titer exceed 1 to 80. This fact, if it prove to be consistent, would indicate that the successful abortion of the disease would depend on the strength of the antibodies that may have been produced in the patient's blood. In other words, if a resistance is already effectively established the additional impulse produced by injecting the vaccine is sufficient to tip the balance in favor of recovery. One might go further and assume that the hyperleucocytic crisis which we have shown occurs in animals and in human beings following the injection of sensitized typhoid vaccines, produces results only when there are sufficient antibodies present in the patient to affect the bacteria that are present in the circulation and cause them to be more readily phagocyted. It is interesting in this connection to note that Pensuti⁴⁶ found that those cases which were unaffected by his vaccine treatment gave a constantly negative Widal reaction. Koenigsfeld²² obtained the best results from auto-serum therapy by using the blood of the patient when the Widal was high. In the cases reported by Sladek and Kotlowski,³⁹ the successful abortive cures were accompanied by unusually high Widals. The beneficial effects of high antibody content in the patient is further indicated by our observations as well as those of Dittborn and Schultz,⁴¹ who found that the most striking results are secured after the 10th day of the disease rather than before, although the older methods of amelioration of the symptoms and shortening of the disease by subcutaneous injections of vaccines state that the earlier the treatment was begun the better the results. Recent experience would seem to indicate that the course of typhoid fever is not markedly affected by the use of vaccines during the incubation period as is evidenced particularly by the cases reported by Elmer⁴⁷ and by Goldscheider and Aust.⁴³ These results are not of necessity in contradiction to the statement of Vincent,⁴⁸ who thought he had prevented the occurrence of typhoid in known cases of infection by the use of vaccines. In his cases no controls were possible.

To *summarize* the history of the specific treatment of typhoid fever: The first twenty years' experience since 1893 with the use of ordinary preparations of typhoid vaccines administered subcutaneously gave some encouragement for the method in the matter of symptomatic improvement, shortening of the duration of the disease, decrease of the mortality, lessening of relapses and complications, with a few abortive cures. The results attained were of doubtful value and inspired little confidence that the true "heroic," specific, treatment had been found. Modern investigations have introduced the intravenous administration of dead or living cultures and particularly of sensitized cultures of the typhoid bacillus. With such methods far more striking results have been realized. Abortive cures occur in a considerable percentage of cases, perhaps in as high as 30 to 40 per cent. If our working hypothesis is correct, these abortive cures are due to the presence of antibodies in a patient who is actively combating the disease and cure is effected by the action of these antibodies on the circulating bacteria combined with a specific hyperleucocytosis produced by the vaccine, particularly when it is specific and is sensitized. The remain-

ing cases might possibly be favorably affected by a combination treatment of sensitized vaccine and a suitable immune serum which would supply the lacking antibodies.

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LABORATORY AND CLINICAL EXAMINATIONS

By GEORGE DOCK, M.D., ST. LOUIS, MO.

THE founding of a journal devoted to the laboratory side of medicine leads one's thoughts to some consideration of laboratory practice. A radical change has taken place in the minds of the profession as regards laboratory work. We have turned a point that often caused professional shipwreck. That point was the belief that laboratory methods were not only apart from, but antagonistic to the other methods of diagnosis. The belief was based upon a fundamental error—viz., that there must be short cuts to diagnosis, and that these were offered chiefly by laboratory methods. Now the urine, now a section of tissue, now a blood spread, lured the hope that by a glance one could tell what perhaps a long and careful study of anamnestic or physical findings had failed to reveal. In truth, there were reasons for the belief, for it often happened that the search was successful to a striking degree—too often, unfortunately for real progress, for with such a viewpoint the name of the disease, or of some disease, was the chief aim of diagnosis. To the superficial diagnostician, as to the layman in daily life, the name seems the great desideratum. Of course the old view will only slowly disappear, more slowly in some places than others, but it is certain that what was formerly a commonplace is now becoming more and more rare. As examples, even now one may occasionally discover myxedema in a patient sent specifically for a urine examination in a case long called Bright's disease, or a leukemia under similar circumstances, neither blood nor urine having been looked at in either case. But it is becoming rare to find a patient long treated for malaria when a physical examination and sputum examination would have disclosed tuberculosis months before, and it is refreshing to find the same improvement as regards septic endocarditis. Ignorance of the conditions of instrumental diagnosis long led to characteristic mistakes and recriminations. For histologic examinations, blood clots or granulation tissue were blindly scraped off from an ulcer or a cervix and much disgust was expressed because early diagnoses of malignancy were not made. For a sputum examination saliva or food remains were presented, notwithstanding careful directions for obtaining the proper material. Obviously, clinical examinations were no more thorough or accurate where such methods prevailed. After explaining, in a public lecture, the absolute necessity and also the value of inspection in suspected pulmonary tuberculosis, the patients brought in for a clinical demonstration were told they need not undress, and as each garment was reluctantly taken off, from overcoat down, at my repeated request, the "cases" were advised by my recent auditors that that was enough. It was no surprise to find in the first patient, one who was a great puzzle in the local medical circles, and whose sputum had often been almost sent to the state laboratory for examination, complete immobility of one side of the chest and other signs of advanced disease. The change of view is due partly to different opportunities for studying medicine, partly to a much larger body of medical knowledge. Patients as well as physicians have come

to realize that laboratory diagnosis is a part of diagnosis in general; that it is capable of giving information of value, often of cardinal importance; that like all other diagnostic methods it varies in value and in certainty in different cases and at different times; that its chief function is not to give names but to reveal details of many and various kinds. Such details are etiologic factors, anatomic or functional conditions of organs, or the biological or chemical constitution of certain secretions or excretions or fluids, all of which go to make up the real diagnosis of the patient's case. An interesting index of the change is found in the fact that some who have been very successful laboratory specialists are preparing themselves for some or other department of practice,—not because they care more for the latter, but because they see a narrowing field of practice for the laboratory. I have spoken of changes in methods of study. For a long time after laboratory methods were available,—the description of tube casts by Henle in 1844 makes a conspicuous epoch, not only physicians long in practice, but even recent graduates, were totally untrained in laboratory methods. Only those who devoted special time and who had special opportunities could do the work, and their aid had to be called upon even for the most ordinary matters, such as albumin or glucose tests, blood counts, etc. Now that practical teaching has been so widely adopted and hospitals and dispensaries give so many opportunities for clinical practice the present generation no longer needs the aid of specialists, but finds it better, for many reasons, to make its own laboratory tests. Moreover, physicians of the older generation, untrained in laboratory manipulations, could not work up newly discovered methods, in their irregular and uncertain leisure hours, even if they understood the new technical terms. Those who have been properly prepared in laboratory manipulations and principles can develop facility in new methods without difficulty, from journal articles or books, and cannot so readily lose touch with the constant advance of medicine.

The change in teaching and in opportunity for practice has exploded a fallacy that hampered many. I refer to the view that some people have no talent for laboratory work. Of course there are differences in natural manual dexterity, in sharpness of vision, in color sense and other particulars, but one who has worked with many undergraduates finds few indeed who cannot become facile in any branch of laboratory work.

Widespread acquaintance with the rudiments of diagnosis can easily be continued and enlarged by all beginning practitioners, none of whom should begin without a laboratory equipment and the determination to enlarge his fund of knowledge and experience, of observation and experiment. Besides the cultivation of special skill, other advantages will follow. The greatest gain will not be the economic one. Far greater in importance will be the earlier diagnosis in its prognostic and therapeutic bearings, and perhaps even more so, the growth of well-founded confidence on the part of the physician.

Laboratory tests, like all others, are often needed promptly. If one must wait for a report even for a few hours, vital time may be lost. So the trained physician will make most of his laboratory examinations, or at least have them made in his office, for much of the work can be made by trained assistants who need not be physicians. Most men will probably wish to see albumin tests, urine sediments, sections and blood smears, as they will prefer to see

eruptions, throats and eyegrounds, to percuss and auscult. But they need not count leucocytes, nor make ordinary titrations, any more than they take temperatures, because such details can safely be left to others and give the physician time for work that he can do better than others.

But the laboratory specialist will continue to have a field, and a more important and interesting field than ever before. Many serologic methods, now so necessary in practice, can be carried out on a large scale as well as on a smaller one. Many chemical investigations can be done while other manipulations are being carried out, and some laboratory workers will develop special skill that may be invoked at times just as do many ordinary clinical methods, and this skill will be kept up and increased by the larger material that comes to the specialist in any line. New tests will often be referred to such experts by physicians who have not time to work at all the methods proposed, although the young physician with spare time will often spend that time in just that kind of work, knowing that all such experiences have a training value comparable to other clinical work. It may even have a relatively greater value, unsuspected at first, as in the case of the X-ray or even the electro-cardiogram. The wide use of the former is obvious, the need of the latter not so well known. In the early days of the string galvanometer it was rather widely accepted that although useful, indeed indispensable for the detection of certain rare cardiac anomalies, its function would for a long time be limited to physiologic research. It soon became clear that although the field for research is still large and inviting, the instrument is capable of replacing all the older instruments used for making objective records of the heart's work, and surpasses them all in the light it throws on many problems of cardiac pathology. Special laboratory work need not be done in private institutions. Hospitals have in the past sadly neglected it, but they are rapidly making up for this and their laboratory staffs will naturally be called upon to do many of the special examinations.

With a general acceptance of the views set forth, a periodical devoted to laboratory methods must have a field as large as that of even the most general clinical journal. It must interest and be necessary to the general practitioner as well as to the special laboratory worker, must be a source of information alike to those who do the work and those who have others do it.

At such a time one who has long been working in the laboratory may be permitted certain comments on contemporary methods. Such common neglects illustrate the incomplete recognition of the use of all diagnostic methods. Let me illustrate this with two examples. At a certain period of the history of medicine it was believed that the finding of elastic tissue in sputum was a valuable aid in the diagnosis of pulmonary tuberculosis, at that time recognized chiefly in its later stages as consumption. After the discovery of the tubercle bacillus that work was abandoned, just as some people abandoned physical examination of the lungs after the discovery of X-rays, and sent their suspects to X-ray laboratories without so much as a glance at the thorax. But the finding of elastic tissue has a distinct and special value. Without regard to its rarity in processes other than tuberculosis, that tissue, usually easy to recognize, is readily found when present, and indicates with absolute certainty a breaking down of lung tissue. This isolated fact should be of a value comparable to that furnished by the thermometer or the blood counter, and should

therefore be sought by all who wish to have accurate knowledge of their patients. The other example is the detection of myoidema. This also was once heralded as a sign of consumption. It would be idle to consider it in that way now, but as a sign of a peculiar condition not yet understood, it deserves further attention in all cases in which it occurs, just as the more popular skin and serum reactions. One can often see the necessity of learning the relative importance of diagnostic methods and their practical application. This can best be done by critically noting one's results, but some fundamental principles may be emphasized. Examinations should be as complete as possible, but just as one cannot, for example, note the details of hair in all cases without the danger of overlooking something more important, so can similar things happen in laboratory practice. As one must observe the hair in cases of possible ductless gland, so in certain cases the rarer organic compounds in the urine must be worked out. But more than once I have known men to make quantitative tests of many rare urinary elements and fail to note a pyuria or bacilluria. Certain venerable errors should cease. An interesting book might be written about the mistakes in the microscopic examination of stools. There are some troublesome elements in many stools, but surely the noting of vegetable tissues such as spiral ducts or cereal hairs for parasites should not continue, now that all medical students have to study the elements of biology. Among the many errors in this respect that have fallen under my observation one of the most pathetic and remarkable was that of a middle aged physician whose life was made miserable for years by the diagnosis, repeated by many men in various parts of the country, of spiral ducts for hookworms.

Another common error in laboratory diagnosis is in expecting finality when the conditions do not warrant it. One still sees a diagnosis of typhoid rejected, and inferior treatment carried out because a Widal test is negative. This is all the more unfortunate because it has been recognized for a long time that the Widal reaction is not to be looked upon as an early sign of typhoid. Many other negative results are wrongly assumed to be final, such as the absence of tubercle bacilli on a single examination, the symptoms continuing. One of the great advantages of laboratory methods is that having certain data established, one can put the patient on a physiologic treatment and follow up the progress of the case with the necessary laboratory tests. This is especially true of some obscure blood disorders and is applicable to many cases with renal alterations, while in glycosuria it finds its most valuable if not its most brilliant field.

Another point often misunderstood is the place of the laboratory test in the course of the examination. Following many formal schemes for case taking, the laboratory work is usually done last, though if specimens are available there is no reason why they should not be examined at once. In many cases the further work is much simplified by making the laboratory examinations first. If positive, the rest of the examination can be developed on more accurate lines than otherwise; if negative, that information is utilized so far as conditions warrant, and the examination proceeds in the usual way.

Not saving of time, or of thought, is the primary object of any diagnostic method of value, but the more complete and accurate knowledge of the patient's condition. The fuller and more exact the diagnostic data, the more certain will be the treatment.

ON THE PROBABLE TOXIC EFFECTS OF PROLONGED ADMINISTRATION OF PARATHYROID GLAND

BY ROGER S. MORRIS, M.D., CINCINNATI, OHIO.

THE effects of prolonged administration of powdered parathyroid gland are so imperfectly described that the following case seems worthy of note,—the more so, as it possibly sheds light on the clinical manifestations of hyperactivity of the parathyroid gland.

A middle-aged married woman was referred to me more than two years ago. For a number of years she had suffered with paralysis agitans.

There was nothing of importance in the family history or in the patient's past history.

The present illness had begun insidiously several years ago, as already stated. Various therapeutic measures had been tried without success. Some twenty months before the patient came under my care, fresh parathyroid gland of the ox was administered daily for several weeks. Then, about eighteen months before I saw her, this was discontinued, and she was given Armour's powdered parathyroid gland, grain I, in capsules thrice daily. Apparently, there was improvement in the patient's symptoms for some months.

After eleven or twelve months of parathyroid extract administration, the patient is said to have begun to fail. However, the powdered gland was continued uninterruptedly, until she came under my care. The history during this time, as I have been able to obtain it, is unsatisfactory, for the patient's recollection of recent events is dim, and for a part of the time her memory is a blank. From the nurses' bedside chart, the greater part of the data is obtained.

For at least three months, the patient's mental state had been abnormal. She was oriented in regard to place but had no idea of time. There was apparently great indifference to surroundings; she took little or no interest in what happened. She usually refused to answer questions, though there was no interference with speech or hearing. Her memory was poor. She often refused food or medicines; for a time it was necessary to conceal the medicines in the nourishment. When the patient did talk, speech was often incessant for an hour or more, and usually what she said was irrational. She was frequently irritable and cross. In the night, especially, she was apt to sing for an hour or so at a time. For many months she had a purposeless habit of knocking on the wall.

Auditory hallucinations were present. No visual hallucinations were noted.

Insomnia was one of the earliest symptoms to make its appearance, when the patient began to fail. It is impossible to give any definite statements previous to the time the special chart was begun. For three months the amount of sleep the patient had has been accurately recorded by the two nurses who were constantly with her. From their notes, the accompanying chart has been made. From this it is evident that the patient's sleep never amounted to as much as seven hours, except the first day charted. On this occasion the patient

slept only three hours during the night, but had five hours' broken sleep during the day. The longest period of uninterrupted sleep the patient had during the first two months charted was about two and a half hours. She was always very restless and wakeful.

Motor unrest was pronounced at times. The patient would toss about in the bed, pitching her body and throwing arms and legs. Because of this, it was necessary to have someone with her constantly, to prevent the patient's falling from her bed.

For three months or more, the patient urinated and defecated in bed. There was apparently no loss of sphincter control, as subsequent events seemed to show. Rather, the patient was quite indifferent to what happened. Her language and manner were at times coarse, quite at variance with the charming, sensitive, highly refined individual she proved to be.

Much of the time the patient had tachycardia, the pulse being between 100 and 106, weak and irregular, as recorded on her chart. The blood pressure was not measured until seventeen days after discontinuing parathyroid; then it was 120 m.m. Hg. systolic. Menstruation occurred regularly at the normal interval of time. There was rather marked anorexia.

At my first visit, I found the patient lying in bed in the dorsal position. She seemed quite unconscious of those about her, and from time to time made brief comments, which evidently related to auditory hallucinations.

The patient was well nourished. The color of the skin and mucous membranes was good. There was no enlargement of the lymphatic glands; the thyroid gland was not enlarged; there was no thrill or murmur over it. The pupils were equal and reacted actively to light and accommodation. The patient refused to co-operate in attempts to test the extra-ocular muscles. The tongue was moist and clean, the teeth in fair condition.

The lungs were clear on percussion and auscultation.

The heart apex was neither seen nor felt. By auscultation the sounds were loudest in about the normal position. Relative cardiac dullness was not increased. The sounds were rather weak but clear. The pulse was regular in force and rhythm, 24 to the quarter minute, rather small in volume, of fair tension. There was no thickening of the vessel walls.

The abdomen was about on a level with the ribs. Liver and spleen were not felt; by percussion neither was enlarged. There was apparently no tenderness on palpation, and there was no rigidity.

There was a small bed sore over the sacrum.

No tremor was noted in the extremities. The tonus of the muscles was distinctly increased.

The biceps and triceps tendon reflexes were present. The knee jerks were present and were not increased; the plantar reflexes were normal. There was neither patellar nor ankle clonus. The abdominal reflexes were present.

Sensation could not be tested satisfactorily.

During the course of the examination the patient defecated in bed. She seemed perfectly unconscious of the fact. It was soon evident, however, that the passage of urine and feces in the bed was not due to loss of sphincter control—for the patient asked for the commode at times and used it,—but rather

to her mental state. Urine was normal. The blood was also negative. A differential count was made; it was normal. The record was unfortunately lost.

Within two days after my first visit, parathyroid gland was discontinued, and the patient was given tincture of nux vomica before meals, veronal and cold sheet packs at night. The second night, after discontinuing parathyroid gland extract, the patient slept seven and a half hours with the aid of $7\frac{1}{2}$ grains of veronal. This was the best night's sleep she had had since the bedside chart was begun three months previously. Within a week, involuntary defecation ceased entirely, and after two weeks, the patient no longer voided urine in bed. The tenth day after opotherapy was stopped, the patient slept two and a half hours during the day and seven and a half hours during the night without veronal, which was then discontinued. In the two years which have elapsed since then, she has slept well and has rarely required drugs. The bed sore healed promptly.

For about two months after parathyroid extract was stopped, the patient

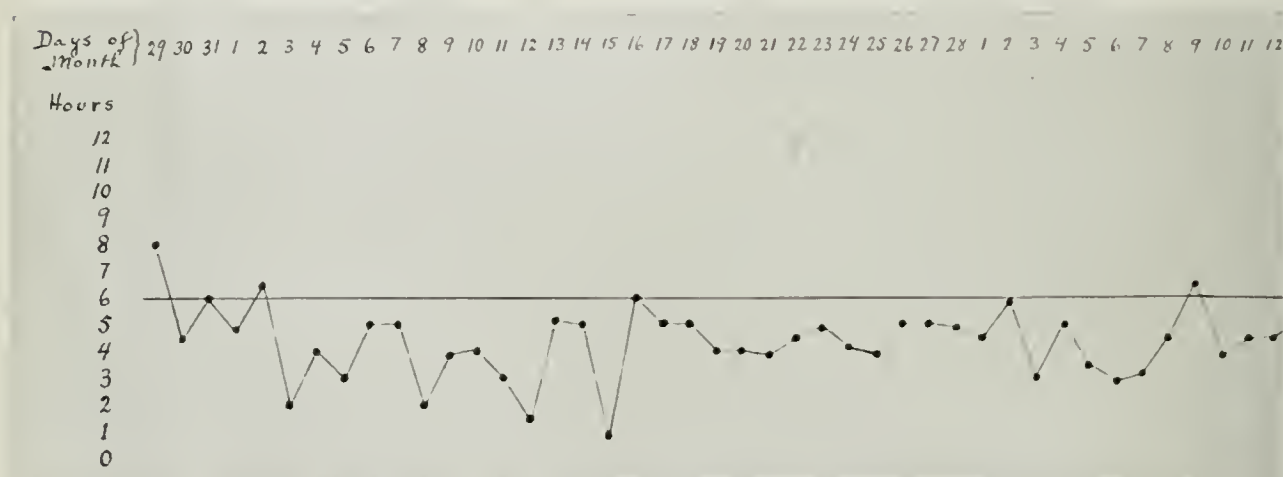


Chart showing the amount of sleep daily.

remained abnormal mentally, though she showed steady improvement. She was very willful and obstinate and insisted on having her demands granted. She often exhibited childish retaliation when not allowed to have her way.

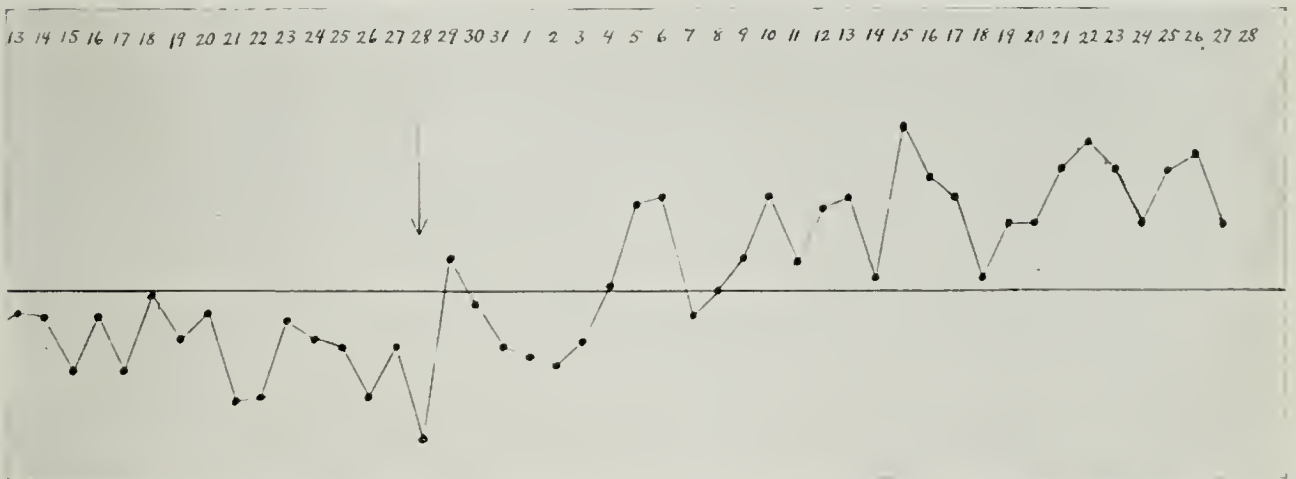
Seventeen days after parathyroid extract was withdrawn, the patient was wakened from sleep by a convulsion of short duration, which involved chiefly the flexors of the extremities, the nurse reported. It lasted only a few seconds. A more accurate description could not be obtained. The next morning when I saw the patient, physical examination showed nothing more than previously. Thinking that the convulsion might possibly have been due to the discontinuation of the parathyroid gland, Trousseau's and Chvostek's signs were tried; each was negative. Nevertheless, the patient was put on calcium lactate and was given one grain of Armour's parathyroid gland substance every third day. There has been no recurrence of the convulsion.

The mental condition of the patient finally became normal, nearly three months after parathyroid was stopped. She said she had no recollection of events for six months, including the first two months of her convalescence.

The tremor became more pronounced ten days after stopping parathyroid gland substance and has remained so.

The evidence points strongly to the view that the symptoms narrated above were due to the powdered parathyroid gland which the patient received for eighteen months. The promptness with which recovery began after the gland was withdrawn is suggestive. Furthermore, in the text-books which I have consulted on Parkinson's disease, I have been unable to find a description of symptoms similar to those my patient suffered from; it is certainly not a usual complication of the disease.

The dosage of parathyroid gland is very indefinite. I have been able to find practically nothing in the literature. It would seem, however, that my patient received liberal quantities, for she was given one grain of the powdered parathyroid gland (Armour's) three times a day. This is so prepared that one grain of the powdered gland is equivalent to six grains of the fresh gland. When one recalls that the total weight of the four fresh parathyroid glands, in man, is usually 0.14 gram, according to Thompson,¹ it would seem that the equivalent of 18 grains or 1.2 gm. of *fresh* ox parathyroid per diem would be an over-



The arrow indicates the day on which parathyroid administration was discontinued.

dose. Indeed, it seems surprising that unpleasant symptoms did not occur sooner after beginning the treatment.

A description of toxic effects from the administration of parathyroid gland, I have been unable to find in the literature. Berkeley² refers to it very briefly in discussing the treatment of paralysis agitans with parathyroid nucleo-proteid, prepared according to Beebe's method. He says, "I have had but one or two patients out of the hundred or more in my own care who really had an idiosyncrasy for ox-parathyroid. These could not take it in any form or dose without such disagreeable by-effects—'nervousness' and insomnia—that it was impracticable to continue the medication." Brief though it is, this would seem to have an important bearing on the case reported above, in which insomnia was so striking.

Salvioli and Carraro,³ in an experimental study of the effect of watery extracts of ox-parathyroid gland on the circulation, reported a fall in blood pressure following intravenous injections, which was not due to local action on the vessel wall, nor was it from action on the vasomotor center. They concluded that parathyroid extract acts as a poison on the heart muscle, whose strength it lessens. This observation may possibly throw some light on my

case. During the height of the supposed toxic symptoms, in addition to the moderate tachycardia already noted, there are frequent entries on the bedside chart describing the pulse as "weak" or as "irregular." The pulse was small and weak, but I was unable to detect any irregularity, though it must be recalled that the patient received the extract less than two days after I first saw her.

As the symptoms from which the patient suffered were chiefly mental, it is impossible to reproduce the condition in experimental animals. Similar experiments in man are, of course, not to be considered. As a result, the interpretation of the case, as I have outlined it, must remain in doubt for the present, at least. It seems possible, however, that somewhat similar clinical syndromes may eventually be found to be due to overactivity of the parathyroid glands,—in other words, to hyperparathyroidism. Should such prove to be the case, it would lend support to the supposed toxic origin of the symptoms in my patient.

It is a very great pleasure to acknowledge with sincere thanks my indebtedness to Dr. W. S. Halsted of Baltimore for his generous assistance to me in searching the literature.

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PRECANCEROUS CONDITIONS OF THE SKIN

BY MARTIN F. ENGMAN, M.D., ST. LOUIS, MO.

HISTOLOGICAL investigation of all precancerous conditions points to primary or preliminary changes in the corium and its vessels before there is any obvious proliferation and downgrowth of the epithelium. These preliminary changes in the cutis seem to be brought about by various clinical factors which may be divided for study as follows: (1) Senility; (2) Actinism; (3) Chemical trauma; (4) Mechanical trauma; (5) Chronic inflammatory diseases.

1. *Senility*.—Little is known of the anatomical and chemical changes coincident in the senile skin. No standard has been established as to what should be considered senility of the skin. Both the microscopic and macroscopic appearance depend so much upon external and internal conditions that it is almost impossible to establish such a standard without numerous controls in its study. In fact, senility is such a broad term and includes so many conditions that are taken for granted, as being due to the natural wear and tear of life, that it is probable that many at present unknown remediable conditions are included in this process. Senile changes in the body may begin at any age, but they are particularly early in those whose mode of life subjects them to exposure to the elements and whose subsistence is obtained by hard toil.

The physical changes of senility are probably first observed in the exposed parts, particularly upon the face and hands. The progress of the change is somewhat different in the two locations. The first changes occur on the backs of the hands in the form of unnatural freckles, the earliest sign being the persistency of freckles upon the forearm and hand. In those in whom senility is beginning, the freckles persist from one season to another; they are larger than those usually seen in youth and are of a deeper color. Upon the backs of the hands only a few may persist and become organized, as it were,—that is, they become slightly raised above the surface of the skin in the form of flat papular lesions which may be smooth or be covered with horny or friable scales. In the beginning of senility, such scaly lesions generally undergo involution, but later they are more persistent and remain for a longer period of time. As years progress, they become still more permanent and may develop into wart-like excrescences, or remain as flat scaly lesions which extend peripherally to various dimensions; not infrequently they degenerate into carcinoma. Some of them become deeply pigmented¹ and are seen upon the hands as very dark or brownish scaly plaques or warty growths. Coincident with or preceding the pigmentation and the formation of the warty or scaly plaques, atrophy begins in the skin and its appendages. As the process develops a change in the pigmentation and character of the skin occurs over the whole surface of the back of the hands; the skin becomes yellow and wrinkled, and when taken between the fingers, seems to be almost as thin as tissue paper; the blood vessels showing through as if the skin was almost translucent. Cheattle,² quoted by Crocker,

¹The Precancerous Melanoses of Dubreuilh.

²Cheattle: Allbutt's System of Medicine, vol. ix, p. 612.

refers to this condition as "biotripsis" or "life wear," and although he states the condition is due to exposure, yet it may be seen in aged individuals who have not been exposed to the elements.

Senile changes of the skin of the face make their appearance later than those upon the hands. They are introduced by some wrinkling and change of contour about the angles of the lower jaw, which progress upward over the face. An erythematous blush over the seborrheic areas sometimes occurs quite early, followed by pigmentary deposits, particularly over this region and near the hair line. The pigmentation occurs as dark or yellowish deposits; some of them seem to be buried deeply in the skin as black or yellow plaques. These dark, pigmentary lesions occur most frequently about the temporal regions and over the cheeks.

Independent of the pigmentary change, greasy, scaly plaques occur over the seborrheic areas of the face and chest, in the form of wart-like growths or horny projections, in which the scales may be friable, greasy and yellow, or of a dirty blackish color, and are easily removed with the curette. When the horny or friable scales are removed from these lesions a bleeding surface is exposed, covered at its edges with a soft necrotic material which is easily scooped out. Such lesions are called *keratosis senilis*, *verruca senilis*, or *seborrheic warts*, and are very frequently the site of malignancy. Following the pigmentary and keratotic changes, atrophic spots soon supervene. They occur most frequently about the sides of the cheeks and the lower lids, and are often the cause of *ectropion*. *Telangiectases* and *nævus-like* lesions soon appear, following atrophy, and even before the atrophic changes are marked, they may be scattered irregularly over the seborrheic areas of the face and chest. Therefore, in the more marked cases, senility seems to give the patient almost the appearance of one suffering from *xeroderma pigmentosum*.

Senile changes progress more slowly upon the covered portions of the skin, but frequently there are found upon the back and chest, senile warts in great numbers, which usually attain to a larger size than they do upon the exposed portions; many of them having the clinical appearance of *nævi*, and in many instances late appearing cell rests. In individuals whose occupation constantly exposes them to the strong sunlight and the action of the elements, senility begins earlier in the skin and is quite marked in all of its various clinical manifestations, and therefore in these individuals in whom such active changes are progressing carcinoma is more prevalent. Unna has given the name "sailor's skin" to those senile changes apparently induced by exposure to the elements, as he observed them most frequently in those whose life was spent upon the sea. Similar peculiar changes in the skin are seen in cab drivers, farmers, laborers, and others whose history shows exposure to outdoor influences.

Senile and pigmentary changes occur in all individuals coincident with old age, no matter whether they have been exposed to the elements or have led sedentary lives. In the latter, these manifestations occur much later in life than in those who have been exposed to the weather. It is well known that in all scaly conditions upon the senile skin, independent of the cause, there is a marked tendency to malignant infiltration by the epidermis.

The histological study of the senile skin is interesting, especially when one uses the tinctorial dyes of Unna, which demonstrate conclusively early degenera-

tions in both the connective and the elastic tissue elements. The beginning of senility of the skin can be demonstrated by a change from the normal reaction of these tissues to these dyes.³ There is, likewise, a progressive atrophy of all the elements of the skin and its appendages, which accounts for the falling of the hair, the dryness of the skin's surface, and its curious wrinkled, yellowish appearance. Nutritional disturbances occur early in the process, no doubt due to degenerative changes in the cutaneous vessels, and it is very probable that if histologically examinations were properly controlled, the vessel change could be demonstrated to be the forerunner of the nutritional and degenerative processes in the skin. If one of the small keratotic lesions be neucleated and examined microscopically it would be found to consist of an increase of parakeratotic cells which force themselves downward into the cutis, dipping with finger-like downgrowths between the papillæ of the corium. The lesions are not inflammatory, but there is a certain amount of round cell infiltration about the invading epithelium. The upper epithelial portion of some of them is quite horny, giving them the clinical appearance of warts, while in others, the outer cells are parakeratotic, retaining their nucleus and sponginess, therefore forming a more irritable and scaly surface. In the latter type, the scales are usually more or less greasy, containing more oil, giving to the surface a greasy, seborrheic element, which is no doubt due to the atypical, physiologic cycle of the parakeratotic cells. Such lesions may start from a small point and increase their dimensions by peripheral extension into rather large, scaly plaques or wart-like excrescences. It is very suggestive to watch the development of these lesions from the smaller types to the larger, so much so, that one feels secure in assuming that this is the earliest stage of malignancy; and it does not seem to be a great step, morphologically, between the invading peculiarly changed epithelium of a seborrheic wart and the malignant process that has seemingly sprung from it; but biologically, it is a chasm, as we have still to determine what is the element or elements which cause these invading cells to infiltrate.

Only in comparatively few of the invading warty lesions does malignancy occur, and in this respect we are in the same position as Cohnheim, when he looked about for a stimulus to explain the malignant step that occurs in his "embryonic rests." In fact, Pollitzer and Unna believe seborrheic warts to be only "seborrheically degenerated, soft nævi." Downgrowth of epithelium does not mean a beginning of epitheliomata, as one would seem to gather from morphologic studies by various writers upon this subject. The various anatomical changes produced by the invasion of such proliferating epithelial cells, as one finds in all such growths, are accidental in many of their phases, as such a mass of cells may invade the mouths of follicles and push aside certain elements of the cutis, causing certain reactionary inflammatory changes. Histological evidence does not disclose the vital step that has occurred biologically in these growing cells; the agent that has caused them to infiltrate and destroy the surrounding tissue. In many of the non-malignant downgrowths of the epidermis, one may find nests of cells which attempt to physiologically functionate and form whorls, but even this does not signify that they are malignant. And the same peculiar clumping of the chromatin, changes in the cell-walls, atypical mi-

³Himmel: *Arch. f. Derm. u. Syph.*, 1903, p. 47.

⁴Kreibeech (*Arch. f. Derm. u. Syph.*, 1903, p. 325) found fatty degeneration of elastic fibres similar to that found in arteriosclerosis.

tosés and the various other morphological microscopic features which are said to be significant of precancerous change are also found, even more frequently, in other pathological conditions of the skin which rarely if ever becomes cancerous.

2. *Actinism*.—Light is supposed to cause the change in the skin seen in xeroderma pigmentosum, and we know that the X-ray causes certain changes in the skin similar to those seen in that disease. We have also seen that the senile skin is similar in its clinical manifestations to xeroderma pigmentosum, therefore, it is safe to assume that the ultra-violet rays of the solar spectrum have much to do with the production of the senile skin in certain individuals. It may be possible that such rays activate some element in the skin which through its influence causes these degenerative and atrophic changes. But if this be due to chemical change induced by light, it is only in certain individuals that it occurs. Xeroderma pigmentosum is a pre-senile condition and may occur at any age; but unfortunately we do not know the active underlying factors, merely the physical changes which mark the progress of the process by the production of pigmentation, which is, no doubt, an effort of nature to protect the more delicate elements of the skin from the light rays. The dark skin of people of the tropics suggests this, and therefore when we see pigmentation following such exposure to light, we naturally assume that the pigmentation is formed in an effort to protect. Unna believes that such superabundance of pigment acts pathologically upon the skin, which may be true and explanatory of some of the changes induced in this class of diseases. The skin of one who is suffering from the sequellæ of repeated attacks of X-ray dermatitis is similar in its clinical symptoms to xeroderma pigmentosum and to the skin of the very aged in whom pigmentation, keratosis, and atrophy are quite marked. Hyde and various other writers wish to attribute to the actinic rays of the solar spectrum a marked pathogenicity in the production of carcinomata of the skin. They assume that it is only in a certain hypersensitive proportion of individuals in whom this action occurs: "Pigmentation, telangiectasis, atrophy, keratosis, and cancerosis of the skin occur in adults much more frequently than in childhood, reaction to the play of actinic rays of light upon the surface being generally determined after the middle periods of life have been reached." (Hyde.)

3. *Chemical Trauma*.—In certain individuals, whose occupation exposes them to the irritating influence of certain chemicals, there occur inflammatory conditions of the skin which are frequently followed by malignant change. Chief among these is the so-called "chimney sweep cancer" which occurs most frequently upon the scrotum, induced by certain elements in the soot, which constantly irritate the skin. Schamberg has suggested that this may be due to an actinic principle contained in the soot. The so-called "kangri" carcinoma occurs in the natives of Kashmir who wear a small earthenware stove, called a "kangri," attached to a belt beneath their clothing: the heat or friction from this stove irritates the skin and causes a chronic dermatitis, which in many instances ends in cancer. Analine workers and those who work in paraffin, tar, pitch-blend, and various chemicals which cause irritation of the skin, are subject to chronic dermatitis and wart-like growths, which frequently develop into carcinoma. Arsenic when taken over long periods or accidentally absorbed

into the system over long periods produces a hyperkeratosis and pigmentation of the palms and soles, which not infrequently develops into malignant infiltration.

4. *Mechanical Trauma*.—Mechanical trauma also may produce changes in the skin at the point of continuous irritation sufficient to induce malignant infiltration of the epidermis. We see this on the lip as the result of constant irritation by pipe stems and cigars, and in the mouth from the irritation of the hot smoke striking continually upon the same point; also in the betel nut chewers in Africa, particularly in the women, who rest the nut against the side of the cheek. A similar condition is caused by the tobacco plug in tobacco chewers. Various other sources of trauma, such as ill-fitting dental plates, especially in certain occupations in which constant irritation causes chronic dermatitis, frequently determine the site of malignant change, as pointed out by Wolbach in cattle, in which traction is made over the horn by a rope, causing at the point of constant irritation an inflammatory area which is followed by carcinoma.

5. *Chronic Inflammatory Diseases*.—In fact, all of the precancerous conditions present in the beginning, chronic inflammatory changes in the cutis, but what we wish to call attention to under this heading is the carcinomatous change which not infrequently occurs in the chronic ulcerations of syphilis and tuberculosis, in old patches of psoriasis, lupus erythematosus, lupus vulgaris, chronic ulcers of the leg, in Darier's disease (Wende), in old irritated, hypertrophic scars, burns, and in various other chronic inflammatory diseases of the skin. In fact, one must realize that in every one past middle life or even earlier, malignant change may occur in patches of skin that have been subjected to prolonged irritation from inflammatory processes, no matter what may have been the source or cause of the inflammation. Leucoplakia of the tongue or mucous membranes of the mouth, inflamed tonsils, various keratoses of the tongue and mucous membranes of the mouth, or chronically inflamed teeth, at points of irritation from badly fitting dental plates, are frequently sites of malignant change. Therefore, in such chronically inflamed patches one must be on the lookout for pearly epithelial borders, for dark greenish crusts, for the heaping up of epithelial cells on the surface, for increased infiltration, for beginning ulcerations in patches that were formerly not ulcerated, together with some infiltration and pearly condition of the border, as these symptoms are indicative of malignant infiltration.

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The study of precancerous conditions just given conforms suggestively to the theory of Ribbert. All of them give microscopic and clinical evidences of long continued disturbance of the cutis (the connective tissue), the supporting framework of the skin, accompanied by various forms of abnormal cornification, parakeratosis and less frequently hyperkeratosis with peculiar changes in the nuclear body, chromatin and cell wall, so called degenerative processes.

With Wolbach, we cannot help leaning in these instances to the theory of von Hansemann, who believes that "the tumor cell is one that has suffered modification, characterized by a loss of the power of differentiation and the acquisition of increased powers of growth." In all of the instances of carcinomatous change occurring in inflammatory conditions, the irritation or in-

flammation has preceded the malignant change by a number of years. In other words, it seems necessary before malignant change occurs in these instances, to have a prolonged disturbance in the life-cycle of the cells of the part or site, where malignancy occurs. Here is where Ribbert's theory is somewhat applicable: a disturbance of the physiological functions of the epithelium induced by the prolonged change in their cycle of growth preceded or followed by snarling off of a portion of the invading interpapillary pegs by the connective tissue. At this time the cancer may be considered "to be a parasitic individual engrafted on a normal individual," and that they are produced by the conjugation of cells in a "way anomalous to the conjugation of sexual cells which produce a normal individual." (Nichols.)⁵ It is interesting to note here that carcinoma has been produced in a rat by prolonged X-ray exposure, which is possibly the only experimentally produced carcinoma on record. Wolbach in the study of X-ray cancer maintains that the acquisition of malignant powers is only completed during years of active proliferation, accompanied by progressive impairment of nutrition, as all of the carcinomata occurring in X-ray dermatitis supervene only after the dermatitis or inflammatory process has existed for years.

It is curious to note that in Wolbach's investigations, possibly the earliest changes seem to occur in and about the cutaneous vessels, which is the case in all precancerous conditions. There is in all of them early evidence of nutritional change, which probably disturbs the physiological function and the life cycle, of first, the connective tissue cells, followed much later by those of the epidermis—a tissue composed of cells of a higher type, whose functions are physiologically much more highly differentiated and whose biological characteristics render them more susceptible to all forms of irritation. They are therefore more easily sensitized to chemical and mechanical influences, as is demonstrated by the various peculiar epithelial reactions to external and internal stimuli seen in many non-malignant and malignant diseases of the skin. The biochemistry of all cells is greatly influenced by environmental conditions; therefore, the epithelium of the exposed portions of the skin and mouth are constantly subjected to these influences, which may explain the great frequency of cancer in exposed locations.

Constant interference in the normal metabolism and physiological functions of a cell will ultimately produce a cell physiologically and sexually atypical, which seems to be characteristic of the cancer cell when compared to the normal kindred cell of its kind. This is what probably occurs in all the precancerous processes, and has given to them the descriptive title of "Precancerous Keratoses."

*Precancerous Keratoses.*⁶—Under this term we have classed all of the above mentioned clinical conditions which seem to be followed in rare instances by certain distinct and definite morphological changes in the epithelial cells.

The precancerous keratoses occur so frequently after or are coincident with

⁵For theory of Farmer, Moore and Walker and an excellent work on "Tissue and Its Relation to Cancer," see Nichols (Jour. Med. Research, Jan., 1905).

⁶It is interesting here to note that according to the researches of Kreibech (Arch. fuer Derm. und Syph., vol. cxvii, No. 3), the melanoblast of Ehrman or chromatophore of Ribbert, the cells responsible for the melanotic pigment of the skin, are derived from the epithelial cells which have altered their morphological and physiological characters on the impulse of some stimuli. He believes that any epithelial cell may become a melanoblast and that it is identical with the naevus cell and the cell of the melanoma and possibly Paget's disease.

these conditions that they must be, at present at least, accepted as etiological factors, yet their exact causative relationship or influence in this respect is not known. However, we have the so-called precancerous changes occurring in the epidermis and producing distinct objective clinical entities without any apparent local contributing etiological factors. The principal member of this group is the clinical condition known in literature as Paget's disease.

Paget's Disease (malignant papillary dermatitis).—There are several types of this peculiar disease which shade gradually into one another. Sir James Paget was the first to describe the condition. As his cases occurred about the nipple region, a similar process in any other portion of the body caused some confusion in the proper understanding of the process. Since Sir James Paget's original description, many instances of the disease have been reported as occurring in various other parts of the body: the lip, vulva, penis, umbilicus, buttocks, chest and back. In my experience the midsternal, shoulders and back are equally as frequent locations as the breasts.

Clinically, the disease may be divided into a mammary type and an extra-mammary type. The clinical appearance and course differ somewhat in the two locations; also the histological features to a certain degree.

Mammary Type of Paget's Disease.—It is extremely rare, is usually unilateral, occurs most frequently in middle life and begins as a dermatitis-like eruption either on the nipple or the areola and slowly spreads over both parts. The earliest symptom is probably an itching or burning with reddening and distinct thickening of the tissues, accompanied by branny scaling. The process extends slowly, the surface becomes denuded of the horny epithelium, and is of a bright or dusky red color; crusts later replace the scales, and the whole surface takes on a disc-like infiltration with a sharply defined or raised border. The infiltration is distinct and card-like. The denuded portion may be covered with crusts or an oozing, sticky, homogeneous debris. The disease may remain in this stage for a long time, even years, before distinct clinical carcinoma of the breast develops. This is heralded by retraction of the nipple and the obvious formation of neoplasms in the breast itself, or the involved area becomes granular in appearance, ulcerated, greatly thickened and raised, with erosion of the nipple and deep excavated points of ulceration. The disease may extend far beyond the areola. Again, it may begin as a scurfiness and itching at the opening of the milk-ducts with a similar condition scattered over the areola at isolated points. This process extends very slowly until the whole region is involved. Usually a scirrhus form of breast cancer supervenes.

Extra-Mammary Type of Paget's Disease.—This type differs somewhat in its clinical and histological features from the former, but to all intent and purpose it is the same process modified through location. It is very difficult to conform the various reports upon this subject into a succinct description, as writers portray so varied a picture. The most suggestive and valuable article is that of Bowen, who points out the connecting links between the precancerous keratoses.

Paget's disease may be mistaken for a patch of chronic seborrheic dermatitis in extra-mammary locations, but it is a question if such a dermatitis can precede the entity known as Paget's disease. From our knowledge of Paget's disease, this is not probable. No one that I know of has watched the process

in these instances from their incipiency; therefore, when it is first observed by the physician, it has a characteristic appearance. It is always seen as a sharply circumscribed patch (in rare instances patches), situated anywhere on the body, with well-defined borders. The patch has a characteristic dusky pinkish tint, is slightly scaly and atrophic-looking—the whole picture showing a chronic progressiveness. The tendency is to progress peripherally, sometimes more in one direction than in another, giving the patch various shapes. One portion may cicatrize while another extends, or cicatrization may occur in the center. In very large patches where this has occurred, relapse in the cicatrized portion may be seen in the form of a superficial ulceration, a crust or crusts, or small nodules, an attempt at tumor formation. The process is very slow, chronic and superficial as a rule, and does not cause deep or large tumors unless a vital lymphatic region is invaded, like that of the vulva, penis, scalp or breast. On account of the location of the extra-mammary type, there is not the tendency to excoriation of the horny epidermis and oozing as seen in the mammary form. Crusts may form here and there over the surface, yet it is not the rule. A patch is frequently encountered which has an atrophic glistening sunken appearance with slightly raised borders and has almost the appearance of a healed lesion, not unlike that of a very chronic lupus erythematosus.

Extra-mammary Paget's disease is not as infrequent as one would be led to believe from the literature and the comparatively few reported cases. It is probably frequently unrecognized or is diagnosed as something else.

The process is slow and chronic, sometimes undergoes involution, and is very rebellious to treatment. One case of mine, which almost encircled the chest, involved and wiped away the right breast, lasted for 40 years, the patient dying from senility at the age of ninety-three. All portions of the process showed the typical histological appearances of Paget's disease. The breast was surgically amputated twenty years before her death and showed a slow scirrhus process. The left breast was involved years after, but was not removed.

The pathological process in all types is essentially a precancerous one, meaning by this term, one in which the epithelial cells show those well-known morphological changes which are frequently followed by and are often seen in carcinoma. Most authors believe that Paget's disease is from the very first a carcinomatous process, but we have long associated carcinoma only with tumor formation and typical malignancy. We must therefore broaden our view of carcinoma and malignancy and include those clinical conditions which show the type of cell so often seen in malignancy, together with clinical symptoms similar in their ensemble to those produced by certain types of basal-cell cancer.⁷ Then Paget's disease is carcinoma of a peculiar type, capable of producing under certain conditions and in certain localities carcinoma of various accepted varieties. Where the lymphatic arrangement and other conditions are propitious, Paget's disease produces typical, well-known types of carcinoma. For instance, I have seen two cases of a peculiar chronic seborrheic-like dermatitis of the lower lip, with oozing, crusting and some card-like infiltration in old men, which were followed in five and ten years respectively by carcinoma of the glands of the sub-maxillary region. Both cases were absolutely rebellious to

⁷Hartzells' (*Jour. Cut. Dis.*, 1910, p. 388) remarks upon this subject are pertinent . . . "these changes we must regard as carcinomatous, just as we regarded as carcinomatous the overgrowth of the epithelial cells themselves."

all forms of treatment, X-ray included. These cases were undoubtedly Paget's disease of the lower lip.⁸

On account of the peculiar cell degenerations and inclusions found in this disease, Wickham and Darier thought it a disease *sui generis* and due to coccidia. This view has, however, been abandoned and the process is now looked upon as a peculiar type of carcinoma. The old idea that it has the appearance of "eczema" should also be dropped from literature.

Darier cites the following features as portraying its carcinomatous nature:

- (1) Slow development during its earlier stages.
- (2) Minor degree of malignancy.
- (3) Involvement of lymphatic glands only occurring towards the latter part of the process.

The microscopic picture of Paget's disease is characteristic and unique on account of the peculiar "degenerative" (?) processes found in the epithelial cells and the reaction in the derma.

There is an edema of the epidermis which forces the cells apart and causes a swelling of the prickle-layer with distension of the nuclear spaces. The epithelium is increased in depth and pushes itself here and there into the derma. Mitoses are seen in the prickle-layer. The epidermis or horny layer is usually soon lost. The prickles disappear and the cells of the epidermis partake more of the nature and shape of those of the basal-layer as it increases in depth. Cornification goes on in a modified form, and together with the edema and possibly other factors produces throughout the section numerous atypical cells (probably a form of dyskeratosis) which were thought by Darier, Wickham and others to be protozoa. The nature of these cells is now known, and is more or less characteristic of several so-called precancerous conditions. Within a double contoured cell-wall there are peculiar faintly stained granular bodies with the appearance of a limiting membrane; the nuclear chromatin is clumped and the nuclear body peculiar.

The papillary body of the derma is flattened out and studded by plasma-cells, lymphoid-cells and frequently polymorphonuclear leucocytes and mast-cells. These surround the invading epidermis. The vessels are dilated and surrounded by new cells, especially plasma-cells. The process in the derma is of a granulomatous nature with a tendency to organization which causes retraction of the nipple and some of the induration. As the peculiar epithelial cells invade they also infiltrate and spread along the lactiferous ducts and initiate the scirrhus type of cancer, which is really the final stage of the disease.

The danger of the process lies in the infiltrating power of these cells which is so marked in certain regions, particularly the breast, that Jacobæus and others believe them to be gland-cells which have wandered into the epidermis from below, and that the local clinical condition is a result of the previous deep-lying carcinomatous process. Facts do not confirm this view. The extra-mammary type is not so edematous, the lymphatic channels not so widely dilated and subject to invasion; the horny layer is often thickened and parakeratotic and intact. The whole process in the derma is more chronic, not so severe or granulomatous in nature; however, the cells of the epidermis present essentially the same peculiar changes as in the mammary form, except possibly in degree.

⁸Histological material could not be obtained in either case.

STAINING SECTIONS OF LIVING TISSUE, UNFIXED*

BY LOUIS B. WILSON, M.D., ROCHESTER, MINN.

THE fixation of tissues by rapid killing and hardening before further preparing them for the microscope is now universally conceded as a necessary process in the study of many cell structures. It produces variations in the degree of refraction and degree of imbibition of cells and of portions of cells, thus making possible their optical differentiation. It also prevents post-mortem changes in cells, preserving their structures so that they may be studied at leisure. By coagulating fluids and by rendering insoluble certain tissue elements, fixation prevents their removal during subsequent manipulations. By the intelligent choice of proper fixatives and other reagents, we may kill, harden and prepare for the microscope almost any tissue without greatly altering the relative size or shape of its cell elements. The changes in refractive indices and in color reactions, while artificial, are not necessarily misleading though our concepts of the normal relations of cells are no doubt often greatly exaggerated.

While conceding the necessity for fixation in almost all instances for fine cell study, histologists are well aware of the desirability of studying living cells with the least possible manipulation. But living cells are indistinctly marked off from each other, and their complex internal structures have almost exactly similar refractive indices and little or no color variations. Thus, the microscopic examination of untreated living tissues, in other than ultra violet light, quickly runs counter to certain fixed principles of physical optics. Our problem then is the production of variations of color and refraction with the least violence to the cell and without coagulating its protoplasm.

That cells may be stained in the living animal has been abundantly shown by Ehrlich,¹ S. Mayer,² Apathy,³ Bethe⁴ and others. More recently the physiochemical processes by which living cells are stained have been studied by many observers, among them, Kiyono,⁵ Michaelis,⁶ and Evans and Schulemann.⁷ The latter observers have shown that the chemical constitution of benzidine dyes is of no influence on the capacity of the dyes to act as vital stains except as it affects the physical state of the solutions of the dyes. Such dyes are taken into certain living cells by the same forces concerned in the reception of large particles (bacteria, carbon, etc.) into cells, forces based upon alterations in surface tension. The diffusibility of the stain determines only its capacity to reach the cells in a living organism. Its inclusion by these cells is not dependent upon a characteristic of the stain itself, but upon the vital functions of the cells.

Thus, by what has long been known as "phagocytic action," living cells take up certain dyes, but the process is a slow one and the ultra-microscopic particles of "colloid" solutions of the dyes are apt to be so grouped in the cells as to form definite microscopic masses, which may be mistaken for cell structures. This method of true vital staining has great possibilities, and its application is yet in its infancy. Its greatest interest, however, apparently lies in its biologic aspects, that is as an indicator of the differentiation of the func-

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tions of the various cells in the living body. The morphologist, and especially the pathologist making microscopic examinations of operative material and working in as speedy a manner as possible, will find more use for methods based on the principle of staining living cells whose surface tensions are already altered permitting rapid imbibition of dyes.

Many living cells which will not take up certain stains may be made to imbibe them by even so slight an alteration of surface tension as may be produced by shaking the cover glass on the preparation. Tissue freshly removed from the living body is still alive and remains so even after it has been quickly frozen, but the surface tension of the cells of the tissue has apparently been materially altered by the cutting off of the blood supply and by the freezing process. This alteration of the surface tension of the cells permits the rapid imbibition of certain stains, while at the same time the size and shape of the cells may have been altered in only a very slight degree, if at all, by the anæmia and freezing. Theoretically it should be possible thus to find just such a stage in cells, which are dying but yet not dead, that would permit of their differential staining while all their structures were fairly normal in size and shape. Any such method must of course fail both in the temporary preservation of the soluble elements of the tissue and in the permanent preservation of any of the tissue unless there is subsequent "fixation" (that is, coagulation of the proteins). Yet, such a method may give not only quickly obtainable pictures of the normal size, shape and arrangement of the cells, but also important details of the normal internal structure of cells, supplementing, and in some instances correcting, our concepts obtained from the study of fixed tissues.

Ten years ago in seeking some means of rapidly staining pathologic specimens as they came from the operating room that a diagnosis might be given while the patient was still on the table, to guide the surgeon in his further operative procedure, I worked out, from the basis of Bethe's methylene blue intravital staining, a method which has since proved very satisfactory. It has been published twice before⁸ but it may not be amiss to reproduce it here for the sake of those not familiar with it.

DETAILS OF METHOD OF STAINING

1. Freeze bits of fresh tissue, not more than 2x10x10 mm., in dextrin solution and cut sections 5 to 15 microns thick.
2. Remove the sections from the knife with the tip of the finger and allow them to thaw thereon.
3. Unroll the sections with a camel's hair brush or glass lifter in 1 per cent sodium chloride solution.
4. Stain 10 to 20 seconds in Unna's polychrome methylene blue.
5. Wash out momentarily in fresh 1 per cent sodium chloride solution.
6. Mount in Bruns' glucose medium.

The tissue must be fresh, that is, the cells must be still alive, and hence present no cytolytic changes. Almost all tissues which we examine have been removed from the body not more than five minutes, and usually not more than two minutes before they are frozen. However, we have gotten fair preparations in some instances from tissues that have been from one to two hours out

of the body. If kept in the ice chest under proper conditions, they may be stained after a still longer period. *Most failures are due to the fact that the cells are dead before the tissues are frozen.*

The dextrin solution is prepared by stirring dry dextrin into boiling water until the mixture is about the consistency of commercial maple syrup. Five-tenths per cent of phenol may be added in warm weather.

The ether freezing microtome is an unsatisfactory makeshift. Now, that tanks of carbon dioxide may be procured in every town which has a soda fountain, there is no longer any excuse for the use of the ether instrument. The microtome should be a well-made machine capable of cutting to five microns, though sections of many tissues cannot be handled quite so thin. In our experience, the best instrument for the purpose is the Spencer Automatic, though good results may also be obtained with the Sartorius, the Leitz and the new Bausch & Lomb instruments with mechanical knife carriers. A freezing microtome which is in constant service in a laboratory in connection with a surgical clinic receives hard usage and its parts therefore must be strong and well made. The valve for controlling the gas is apt to become quickly worn and leaky. This valve is more convenient if placed at the microtome and not at the tank. It should have a long flat T-shaped handle for ease of operation. Whatever style of microtome is used, the metal plate on which the tissue is frozen should be insulated in some manner from the metal parts of the remainder of the apparatus. This prevents the heat being transferred between the two, makes less gas necessary and keeps the tissue frozen longer.

The carbon dioxide tank should be suspended on metal hooks or brackets underneath the table on which the microtome is placed. Thus, the small amount of dirty water which is present in many tanks will not find its way into, and clog the valve of the freezing chamber, as it is apt to do when the gas tank is placed in a vertical position above the work table.

In freezing, the gas should not be turned on in a large stream and allowed to flow until the tissue is frozen. Much gas can be saved and much more satisfactory preparations made if it is turned on in intermittent spurts, giving time for the tissue to freeze more slowly, and thus more evenly throughout. It should not be frozen more solidly than is necessary for cutting. When this occurs, however, the upper layer may be thawed slightly by placing the finger on the tissue.

The sections should be cut by rapidly repeated strokes, not permitting the surface of the tissue to thaw after each cut. A half dozen or more should be cut, and the first ones rejected, insuring sections of even and uniform thickness.

In trimming out blocks of tissue for freezing, the pieces of tissue should be not more than 2 mm. in thickness, and the transverse diameter should be as small as is possible and still include the desired field of examination. The smaller the block down to 2 mm., the better the sections, and the more easily they may be handled. In general the blocks should be so trimmed that the sections when cut will be rectangular or rounded, rather than triangular in outline. Unless the block is more than 1 cm. in transverse diameter, the long edge should be so placed on the freezing plate that the knife edge will strike it

parallel. Where blocks are more than 1 cm. in transverse diameter, a shorter edge may be placed so as to first come in contact with the knife. When very minute bits of tissue are to be frozen, it is best to partially freeze a small amount of dextrin solution first, and then place the tissue on top of it, thus keeping it 1 mm. or more away from the metal freezing plate and avoiding contact of the knife therewith.

The knife edge must be as sharp as it is possible to make it. The blade should be very rigid, preferably wedge-shaped, and not hollow-ground. We have attempted to use safety razor blades in special holders, but have found them to vibrate altogether too much. Several blades, not less than four, should be provided for each microtome, since the edges are dulled very quickly with the best of tissues, and routine blocks are apt to contain unsuspected calcified areas. Reserve cylinders of carbon dioxid should also be kept in some nearby cool place, so that they may be changed rapidly if necessary.

When sections are removed from the knife with the tip of the finger and allowed to thaw thereon, a process which may be hastened by breathing on them, air bubbles are less apt to form than if they are removed with a brush and placed immediately in salt solution. As the sections go into the salt solution, they are usually more or less rolled up, and must be straightened out by gentle manipulation by moving them up and down either with a small camel's hair brush or with a bent glass lifter with a dull point. This is best done in a clear glass dish over a black background, permitting the unstained sections to be readily seen.

When the sections have been straightened out, they should be caught under the middle with a small bent glass rod lifter and transferred to the stain. If, in the stain, a direct up-and-down motion is made with the lifter, the section will remain thereon, the folded portions merely "flapping" in the stain. Occasionally a section will be lost off the lifter. If the receptacle holding the stain is large, much time may be lost and the section injured in finding it. This may be obviated to a considerable extent by using a very small receptacle, of not more than 10 cubic cm. capacity, with a rounded bottom. Small individual glass salt cellars and the small porcelain teacups from a set of doll's dishes are quite convenient. The bent portion of the glass lifter should be less than $1\frac{1}{4}$ cm. long and the end should be rounded in the flame.

We have never yet gotten an unsatisfactory lot of Unna's polychrome methylene blue direct from Grüber, while at the same time we have gotten one or more unsatisfactory specimens from every other dealer from whom we have purchased, even when the stain was said to have been made by Grüber and re-bottled by the selling firm. Now, that the German supply is temporarily cut off, we are making our own. The secret of success seems to be in taking a large quantity of Unna's alkaline methylene blue (methylene blue 1, carbonate of potassium 1, water 100), and allowing it to ripen for from six months to a year with the largest surface possible exposed to the air in a flask stoppered only with cotton. When the stain is properly ripened, it contains a considerable portion of methylene red, and should give sharply differentiated dark blue, purple and pinkish red color-contrasts with fresh tissue. The stain even in 5 or 10 c.c. amounts, may be used over and over again, but should be discarded when it shows a precipitate or when it no longer gives sharp color-contrasts.

The sodium chloride solution, which is used for washing out the gross excess of stain should be contained in a white porcelain dish, or if in a clear glass receptacle, this should be over a white surface. In this manner, the evenness of staining and the general appearance of the sections may be more readily seen.

Bruns' glucose medium is prepared as follows:

Distilled water 140 c.c.
Camphorated spirit 10 c.c.
Glucose 40 g.
Glycerine 10 c.c.

Mix the hot water, glucose and glycerine thoroughly, add the spirit, shake and filter to remove the excess of camphor which is precipitated on mixing. The solution should be kept in relatively small stoppered bottles. In warm weather a crystal of thymol may be added to prevent the growth of moulds. The solution is cheap, and should be thrown away as soon as it becomes colored with dye.

Sections are most conveniently spread out and transferred to the slide if the solution is contained in a long narrow porcelain dish about 1 inch deep by $1\frac{1}{4} \times 4$ inches. These may be obtained in the market as white porcelain match trays.

The section should be moved about in the glucose medium for a few seconds, not only to straighten it out but also to obtain better differentiation. The end of the glass slide is then slipped under it, the edge of the section held to the slide by the lifter, and the whole preparation raised out of the solution. The excess of glucose medium is wiped off from underneath the slide and around the section, the cover dropped on, and the preparation is ready for the microscope.

All the steps of the process may be carried out in one minute from the time the tissue is placed on the freezing plate of the microtome until the slide is placed on the stage of the microscope. Various tissue elements should be thoroughly contrasted in pink, red, purple and dark blue. Mitotic figures, when present, are beautifully shown. Many bacteria are stained.

The method, as given above, has been used for diagnostic purposes and for the study of fresh tissues in the laboratories of the Mayo Clinic for more than ten years. During that period it has been frequently modified by members of the laboratory staff. All such modifications, however, have been abandoned and the original method returned to. Probably the most useful modification is one which we used several years ago, and which has subsequently been independently developed and published by Pierce.⁹ This consists in the substitution of distilled water for the sodium chloride solution, and of carbol-thionin for the polychrome methylene blue stain. The carbol-thionin stain may be more quickly prepared than the polychrome methylene blue, and is perhaps more stable. Bacteria are stained by it a little more sharply than by polychrome methylene blue. However, sections must not be left over long in the stain which contains $2\frac{1}{2}$ per cent phenol.

Sections stained by the polychrome methylene blue method remain in excellent shape for two or three hours after the tissue has been removed from the body and may be in fairly good condition, if kept cool, for a day or two.

No satisfactory method has as yet been devised whereby desirable sections can be fixed and preserved after staining in this manner. Success in this direction probably lies along the lines indicated in Bethe's original method for the fixation of tissues vitally stained by injections of methylene blue.

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INTESTINAL STASIS AND INTESTINAL INTOXICATIONS: A CRITICAL REVIEW

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

ONE of the most widely discussed questions of the present day is that which concerns itself with intestinal intoxications arising during intestinal stasis. Its period of importance was inaugurated by Bouchard, who gave us that unfortunate term so widely used and so little understood,—especially in this connection,—*auto-intoxication*. Its greatest modern interpreter has been Lane, who has given into the hands of the many undiscerning a series of surgical procedures, awful at their best, but terrifying in the practices of the many who do not discriminate. It is a question on which much is written and of which little is exactly known.

The present vivid vogue of this question as a matter for discussion is more the direct result of the work and writings of Sir Arbuthnot Lane, for he has been able to make it more profitable than Metchnikoff, with all his erudition and experiment. Acting upon the assumption that a very large proportion of the ills from which men suffer are the direct result of absorption of toxic materials from the intestine, he has taught his methods, so that now surgeons (some surgeons) are everywhere seeking to remedy almost any condition by removing ceca, colons, and sigmoids, or all three, or by making unnatural intestinal anastomoses. When one considers the terrifying series of ailments which Lane lays at the door of stasis in the intestinal tract, one is, perhaps, thankful that there is so little evidence of the inheritance of acquired characteristics, or wonders why man was ever allowed a large intestine.

The term intestinal toxæmia, or intoxications of intestinal origin, refers to various symptom complexes which are associated with gastro-intestinal abnormalities of secretion, digestion, and motility. How great such a catalogue may be, one may discern by perusing the following list:¹

1. Loss of fat.
2. Wasting of both voluntary and involuntary muscles.
3. Alterations in the texture and color of the skin with pigmentation and offensive perspiration.
4. Subnormal temperature, especially affecting the extremities. There is no abrupt line of separation between this condition and Raynaud's disease, of which it would appear to be a stage.
5. Mental conditions of apathy, stupidity, or misery, which may become exaggerated to a state of melancholia, or even apparent imbecility, with suicidal tendencies. There may be neuralgic symptoms, neuritis, frontal headache, loss of control over temper. These nervous states, due to stasis, are a much more frequent cause of serious crime than is generally imagined.
6. Rheumatic aches and pains in muscles, joints, and skin.
7. Atrophy of the thyroid.
8. Either increased or lowered blood pressure.
9. Degenerative changes in the breasts, especially in the upper and outer zone of the left breast, predisposing to cancer.
10. Prolapse of abdominal organs, partly because of loss of fat, partly because of wasted muscle fibres; increased mobility of the kidneys, and prolapse and bends of the uterus.
11. Breathlessness on exertion, at times of asthmatic type, due in some cases to a distension of the stomach and intestines.
12. Degeneration of the heart muscle with dilatation of the left heart and aorta and arteriosclerotic changes (atheromatous) in the systemic arteries.
13. Renal changes which are roughly grouped under the term "Bright's disease."
14. Early loss of hair color with falling out, more common in those with dark hair than with red hair.
15. Affections of the pancreas with chronic induration, inflammation, and finally cancer. Pancreatic diabetes.
16. Infection of the biliary system, cholecystitis, cholelithiasis, cancer, together with many acute and chronic diseases of the liver.
17. Degenerative changes of the eye.

All of these are results of absorption of toxic materials from the intestinal tract! All are primarily due to abnormalities of motility!

Watson²¹ is more conservative than Lane. He divides his cases, whose histories are given, into three main groups; one in which the symptoms are mainly those of neurasthenia; one in which the symptoms are those of rheumatoid arthritis; and one which shows, mainly, dyspeptic symptoms.

Leaving to one side the problems which would arise in discussing motility, which is rarely a primary condition, and abnormalities of secretion, let us look at the possibilities connected with absorption from the intestine. They may be grouped as follows:

A. *There is the possibility that during digestion of food materials by the normal secretions of the gastro-intestinal tract, toxic substances are formed and that these substances may enter the blood stream and produce serious disorders.* As a matter of fact, we know that many of the products of protein digestion are toxic.²⁸ The peptones have well-known poisonous properties when they are injected into the blood stream. Even the unsplit proteins, such as egg albumens, are toxic under certain conditions. Vaughan³ believes that the toxicity of proteins resides in the interior of the molecule and that when this portion (its most chemically active part) is set free in the body, it acts as a poison because of its intensely active chemical nature. In the gastro-intestinal tract, if it is set free, it is immediately broken up by the ferments and so becomes harmless. The interior of the body is not constructed for such rapid or complete splitting, and so in the interval which would elapse between its liberation and its destruction, it damages the body and that damage may be shown by such symptoms as malaise, fever, or by such symptom complexes as anaphylaxis. Longcope⁴ by injecting egg albumen into animals has been able to produce morphological changes in the organs, notably in the kidneys, which correspond to the milder degrees or stages of interstitial nephritis. Woolley, DeMar and Clark,^{5 5a} on the other hand, were unable to produce any observable symptoms or lesions by injecting egg albumen, casein or albumose into animals. Longcope's method was to activate his animals to egg albumen and then to produce mild attacks of anaphylaxis by subsequent injections of the same substance. Woolley and his co-workers treated their animals by giving them daily intraperitoneal injections of albumen, casein and Witte's peptone over a period of 40 days. It is possible that had they used Longcope's method they might have produced similar effects. The value of their work lies in the indication of the difficulty that is met in the passage of these substances into the tissues of the animals. Longcope's results indicate what may happen after the substances have entered. Wells,³⁵ some years ago, reported that by subcutaneous administration of Witte's peptone he was able to produce hepatic fibrosis. He, like Longcope, was placing the protein directly in the tissues. Wells now, however, doubts his results.*

It is a long known fact that it is with the greatest difficulty that any of the products of digestion enter the blood or reach the tissues in an intact state, under normal conditions. Somewhere in the progress from the lumen of the gut to the blood, they are so changed as to be non-toxic. There is also another side. Soluble substances of a toxic nature entering the blood tend to produce diffuse lesions rather than focal ones, and diffuse symptoms, rather than focal ones. One would expect, therefore, that soluble poisons such as the peptones and other split-products would produce generalized symptoms and lesions, and that focal lesions and symptoms might be due to localization of bacteria which enter subsequently. Evidence of this has been offered by Opie⁶ who, with chloroform alone, was not able to produce the focal lesions he observed by injecting chloroform and colon bacilli.

*Contrary to many workers, Gibson (Philippine Journ. Sci., 1914, [8, B.] 475) who worked with very carefully purified proteins, which he injected with careful aseptic precautions into guinea-pigs (subcutaneously) says, "It would seem then, that the primary cleavage products of pepsin-hydrochloric digestion, when prepared without drastic treatment, from purified and well-characterized proteins, never have more than a slight pyrogenic effect when injected subcutaneously into rabbits and guinea-pigs. Any temperature rise, if present, is insufficiently pronounced to permit a direct inciting role to be ascribed to such proteoses in the production of the severe naturally occurring fevers." (cf. Jour. A. M. A., 1914 [62] 1835.)

It may be recalled in this connection that Ascoli, Oppenheim and others have shown that proteins fed to animals in large amounts may be demonstrated subsequently in the circulating blood and in the urine, though as Zinsser⁷ says, this happens only under abnormal circumstances. Rosenau and Anderson also have shown that guinea-pigs may be sensitized by feeding horse meat or horse serum, and McClintock and King were not able to confirm it⁷ which may only go to prove that McClintock and King did not encounter the abnormal conditions which made the results of Rosenau and Anderson possible.

The whole general trend of opinion regarding the absorption of protein split-products in a toxic state from the intestinal tract seems to be that while it is possible, it is unusual.

B. *There is the second possibility that bacteria resident in the intestinal tract act upon the food stuffs and produce toxic substances, which are absorbed and act as intoxicants.* Some of these substances belong in the great group of ptomaines, such as cadaverin, neurin, cholin and putrescin; some belong to the aromatic series, as, for instance, indol, skatol, and tyrosin. Para-oxyphenylethylamin is a recently studied substance; aldehydes have been under suspicion, and betaimidazolylethylamin (histamin) is challenged.¹⁸

As in the case of the protein split-products, there can be no doubt that these substances produce grave consequences when they are injected into the organism. There can be no doubt that they are continually entering the tissues through the walls of the intestine. But with exceedingly few exceptions they are so changed in passing the intestinal walls into the tissues, that they are rendered, so far as is known, innocuous. Hervieux²⁹ says that indoxyl is a normal constituent of the blood. Herter was among the first to use these substances in experimental work and his book⁸ has become a classic. He showed that such substances as indol might be toxic, but neither he nor any subsequent writer has brought proof that they ever reach the interior of the body in amounts sufficient to be poisonous. There are two possibilities which would favor the toxic action of these products. One is that because of lesions of the intestinal tract so much is absorbed as to overtax the protective chemical action of the tissues and fluids of the body; and the other is that for some reason the protective combining power of tissues and organs is abnormally low. Concerning either possibility nothing is known. It is known, however, that the healthy body is able to take care of tremendously larger amounts of these toxic substances than are produced, as was shown by Woolley and Newburgh.^{9 10} Iwao,²⁶ however, has been able to produce severe blood changes with minute doses of oxyphenylethylamin, but Charleton²⁷ produced similar effects with *B. coli* injected into rabbits. Under any apparent possibility, however, the absorption would not be the primary trouble, but some other factor which made abnormal absorption possible, and treatment should aim at correction of the primary condition rather than of the secondary.

Metchnikoff¹¹ is a valiant supporter of the bacterial toxic cause of many disorders, notably of old age, but he has never shown but one side, except by inference, in his writings. He has shown that by the use of certain organisms, notably *B. bulgaricus* and *Glycobacter peptolyticus*, the flora of the intestine may be changed, just as other writers^{12 13} have shown that by diet similar changes may be effected. But he has produced nothing except incomplete cir-

cumstantial evidence that the general symptoms associated with an abnormal intestinal flora are due to the toxins or the protein split-products of the members of the flora. The presence of indican or phenol combinations in the urine in abnormal quantities is no sign that indol or phenol are acting as poisons in the organism, it is merely a sign that the contents of the large intestine are not in a healthy condition. Even if it be true, that, as Kendall (p. 136) says, "one is forced to the conclusion that the intestinal tract is a wonderfully perfect incubator and culture medium combined; in this intestinal incubator there is such a range of reaction and diversity of food stuffs that the most varied types of bacteria capable of growing at body temperature can conceivably find a place where the environment is particularly adapted for their development. It must be evident that the direction which the flora takes will not be without influence upon the host," there still are no solid grounds for saying that the absorption of split-products are the causes of symptoms—though they may be.

Distaso²⁵ has reported observations which show that so far as the anærobes of putrefaction are concerned, these organisms play no role in the putrefaction of feces within the body, and also that they perhaps play no role in pathologic conditions.

C. *There is the third possibility that the presence of bacteria themselves, which have entered the blood stream from the intestine, are the source of trouble.*

In support of this it may be said that the bacteria are constantly entering the body from the intestinal tract, and that under certain conditions, the number is increased. These organisms may produce either or both of two effects; they may act as irritants to the cells of the body (more particularly, perhaps, the endothelial cells), or they may, in being devoured by the cells of the tissues, liberate a toxic material which is either an irritant or a true poison. As an adjunct to this conception, it might be added that the soluble toxins of intestinal bacteria may be absorbed and act as poisons to the cells of the organism. In regard to this, one might say that except in very unusual conditions this does not happen, because, perhaps the toxins are modified in passing the intestinal wall exactly as other proteins are.

Adami¹⁴ is the great sponsor of the infectious theory of the origin of fibrosis and other collateral effects. He believes that under certain conditions the usual minimal number of bacteria in the circulating blood is increased and that these produce organic changes. He speaks of the process as sub-infection. Ford¹⁵ and others have studied the bacterial flora of healthy organs. Bierotte and Machida,¹⁶ for instance, in discussing the bacterial content of the organs of healthy animals at a slaughter-house, found 10 varieties of germs in 59.2 per cent of 54 organs taken from 11 animals. An unpublished observation of Dr. Wade Oliver,⁴² working in the Pathologic Institute of the Cincinnati General Hospital, is that the blood taken from the veins of patients, who are brought to the hospital in a state of fatigue, such, for instance, as following a debauch, is apt to show large numbers of organisms, especially ones belonging to the group of hemolytic streptococci. Dr. Oliver believes that the source of these is in the pyorrheic gingivitis from which most of these individuals suffer, or from other foci of chronic infection. Nevertheless, as the physical condition of the patients improves the blood becomes sterile. This observation bears up-

on the intestinal problem merely in indicating the comparative ease with which bacteria enter the body as compared with the difficulty with which non-living proteins enter. All of the recent work on "focal infections"^{17 18} tends in the same direction, i. e., to indicate that the bacteria which are harbored in the body are able under certain conditions of general health to enter the blood stream and to be transported to the various tissues and organs of the body, where they produce focal lesions, and as has been said, it is focal lesions which produce focalizing symptoms.

It seems then that from the standpoint of the literature, the doctrine of infection is the more rational one. Perhaps it were better to say that in the production of symptoms during intestinal stasis the action of bacteria is the immediate cause, but that with this action, after a time, toxic materials may be absorbed through lesions in the intestinal wall produced by the intestinal contents. Accompanying stasis there are certain macroscopic changes in the mucosa which are readily recognized. One of these is a mild inflammatory condition, the others vary from more pronounced inflammatory lesions to ulcerations—such, for instance, as the "Dehnungsgeschwüre." An intestinal wall which is the seat of such inflammations is more permeable to bacteria than a normal one. Under such conditions bacteria and toxic materials pass into the circulation, during which process the latter become, as a rule, fixed, while the bacteria, protected by virtue of their being living, pass on until somewhere or other they become fixed and die, and their poisons are set free.

There seems to be good reason for the belief that many of the suspicious kinks and folds are not essentially harmful, for they are normal, or merely anomalies, and that most of them are of infectious origin¹⁹—the result of peritonitides. But of this more will be said later.

D. The fourth possibility is one suggested by Eppinger and Gutman and is one that has gained little credence, perhaps, because the problem has not been understood. These writers look upon many—perhaps all(?)—of the substances which pass the intestinal walls as hormones, which reaching the tissues produce effects in the same manner as do the active components of the internal secretions,—i. e., by causing them to carry on more actively or less actively, as the case may be, their several functions. They point to the fact that B—Imidazolyethylamin (histamin) produces typical stimulant effects upon the autonomic nervous system, and that para-oxyphenylethylamin (tyramin) a split-product of tyrosin, resembles adrenalin in chemical and functional respects. So they would conceive of the intestines playing a role somewhat similar to that of the ductless glands.

E. A final possibility is that one or more of the previously discussed possibilities occur together.

We were negligent were we to leave the impression that with the five possibilities given above we had exhausted the possibilities in accounting for the symptomatology of so-called intestinal stasis. What we have tried to do has been to make clear the possibilities *from the intestinal side*. We believe that many of the symptom complexes credited to the intestinal conditions have primarily little or nothing to do with the intestinal tract, but that they are the result of infections elsewhere in the body. Lane, for instance, believes that pyorrhœa and oral sepsis are very common in intestinal stasis, as a secondary

occurrence. We believe with Watson⁴¹ that they are primary. We believe, from the writings of Billings,^{17a} Rosenow¹⁷ and others, that when intestinal stasis occurs in conjunction with oral sepsis, it is merely a coincidence and not essential, and that the intestinal condition may become really a serious complication if the stasis is not corrected. Before such a complication occurs the essential place to apply treatment is the mouth. After it has occurred, both mouth and intestinal treatment are essential. In a like manner we believe that the primary difficulty in the rheumatoid arthritis group of cases, for instance, is probably to be sought elsewhere than in the intestine, and though we state this as a belief, yet we feel that there is abundant support of such a belief in the publications of Rosenow and others.⁴⁴ Cases are cited by Watson and others which definitely support such a belief. Even cases belonging in the neurasthenic group of Watson present evidence of the application of the laboratory results of the studies on focal infections in this group. The fact that Woolley⁴⁵ in a case referred to him by Dr. C. A. L. Reed, was able to obtain a pure strain of *B. lactis aerogenes* from a duodenal mesenteric lymph gland, does not mean necessarily that the epilepsy from which the patient suffered was due to that organism nor that the cause of the disorder lay in the intestinal tract—though that may be the truth of the thing. In such cases there is no method advanced for deciding whether the difficulty lies in an abnormal central nervous system which spontaneously reacts with explosions to normal stimuli or which reacts to abnormal (or normal) materials absorbed from the intestine or from some focus of infection in the intestine, or in some other organ. It may or may not be a rather hazardous attempt at prophecy when one describes the morphology and biochemical characters of the unknown microbic cause of a disease like epilepsy.⁴⁶

Associated with these problems of etiology are those of therapeutics. These we may divide into two main categories,—medical and surgical. Within recent years the intestinal surgical treatment has come to the fore, reached its acme and seems now to be declining, the reason for this being that there appears to be a growing opinion that the therapeutic results have not lived up to their promises. It is the opinion of some, perhaps many, physicians, that the Lane operations for intestinal stasis belong in the same class with unnecessary ovariectomies, nephropexies, and appendicectomies.¹⁸

The intestinal surgical procedures include enterocolostomy, or enterosigmoidostomy, colectomy and cecectomy, and plastic operations devised for the removal of bands or kinks, chiefly in the course of the large intestine. The short-circuiting operations allow part of the intestinal contents to go more directly and rapidly to the outside world. It is a sort of flushing operation. Its main disadvantage lies in the fact that the isolation of a part of the intestine results in a condition in which the contents move more slowly and in which the feared fermentations and decompositions go on more vigorously than before the operation. The radical operations have the same effect upon the current in the intestine, but the vicious circle is removed. The cosmetic operation tends in the same general direction except that it tends to ease rather than speed. It straightens the crooked path without shortening it and eliminates the short turns. It is perhaps unnecessary to add here that if something wrong is going on in the bowel which shouldn't go on under normal circumstances,

it will not of necessity be right to remove the bowel. This, at first sight, would appear to resemble what might be done in case something were wrong with the drains of a house. One wouldn't necessarily remove the drain of the house, especially when he might improve or even cure the condition by running some chloride of lime through it. The difficulty with the plastic operations seems to lie in the danger of more adhesions, which are in turn inhabited and perhaps caused largely by bacteria. If, as Archibald¹⁹ found, there are bacteria in the adhesions formed after simple operations in which there is no stasis, what would we expect in cases in which stasis is present? It is interesting to note that the only objection brought by Lane against complete colectomy is the development of post-operative adhesions.

What may happen, as the result of any or all of these operations, is that the feces spend a shorter time in the body and that therefore there is less opportunity for putrefaction, fermentation, dilatation and absorption, or that the bacterial flora is changed, or that both of these things happen.

The medical men, some of them at least, say¹⁸ that they can obtain as good, or better results in the long run, with less hazard. They say that by using the methods of Metchnikoff they can change the flora; by diet alone^{20 12 47} they can modify the flora; by laxatives they can hurry Nature and make her remove the garbage; by antiseptics³⁰ they can kill the parasites; and by mere copious water drinking³¹ they can limit indol production and reduce putrefaction. And, they say, that while using these methods they do not need to worry about the septic tank of the short-circuit operations, the multiplied adhesions of the plastic, nor the mortality, or the adhesions following annihilation of the colon. Also they say that cecal or ileal kinks appear in 18 per cent of all persons¹⁸ and that so many persons do not suffer from "intestinal intoxications." Again, they say that all who are constipated are not ill. Finally some may think as Einhorn believes, and quotes Dunin as suggesting it, that perhaps certain symptoms are not so much the results of constipation as they are causes of it. That there are colons which deserve destruction there can be no doubt, but it is suggested to try everything else first, and if there be no value in diet, exercise, massage, or physic,—use the knife. Eastman³⁷ says "it is probable that time will demonstrate the fatuousness of either short-circuiting or resection operations in cases of stasis not presenting demonstrable obstructing factors," and Lynch and Draper³⁸ feel that "surgery is able to offer in *selected cases* a therapy which often effects a true cure." Dr. Berghausen's work in the Pathologic Institute of the Cincinnati Hospital tends to support treatment (used by Watson and others) by vaccines in some cases of stasis. If this is justified, then there has been added another reason for belief in infection rather than in intoxication.

An editorial writer of the Journal of the American Medical Association²¹ quotes Hale White, as follows: "When these cases are reported we are always assured that all medical means had been adopted without benefit, but we are never told what the medical means were. * * * * A suspicion comes to one's mind sometimes that perhaps some surgeons do not know all the means the physician has at his command for the treatment of delayed action of the bowels." This seems not to be unfair in many instances. Case⁴³ sums the situation up by saying that surgery should not be seriously considered as a cure for ileal stasis

until a most thoroughgoing trial has been made of the various dietetic and mechanical measures at our command.

But again, in discussing therapeutics we have confined our remarks to the intestinal aspect of the question. Washing out the intestinal tract will not cure pyorrhœa alveolaris though it will undoubtedly assist in cure. It will not prevent absorption from a root abscess, or from an area of decomposition under a crown or a bridge. It will not hinder the growth of staphylococci or streptococci in a chronic appendix abscess, or in the partially closed crypts of a tonsil; nor will it drive out the bacteria from the regional lymph glands in a case of arthritis deformans. For such focal conditions the treatment will vary—dentist, physician and surgeon, and perhaps even the bacteriologist, alone or together, may be needed, depending upon the seat of the primary infection.

SUMMARY

By way of summary we may say:

1. That absorption of poisonous materials from a healthy bowel has not been shown to produce symptoms of disease.
2. That absorption of bacteria and other substances from an unhealthy bowel may produce serious symptoms.
3. A surgical operation for intestinal stasis is not justifiable except as a last resort.
4. There is no definite information in the literature to show that surgical procedures, made for intestinal stasis, have been more successful than medical (including hygienic) ones.
5. Many cases in which symptoms are attributed to intestinal stasis are suffering from focal infections entirely outside the intestinal tract. Such infections are illustrated by pyorrhœa alveolaris, chronic tonsillar infection, and chronic infections of the antra and sinuses of the head.

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LABORATORY METHODS

TEST for Albumin in Urine.—Heat and nitric acid, simply and combined, with the contact test, remain the reliable means for the recognition of albumin in the urine. All other tests are objectionable in some respect, and the findings, with them, are subject to misinterpretation. The tests for albumin should be made, when possible, with fresh urine. It is customary with some insurance companies to have the urine sent to a central laboratory. Boric acid is added to the urine as a preservative, and is probably as good as any, but many of the samples when examined, contain more or less of bacterial growth. It is the rule to heat such urines with strong alkali, filter and test the filtrate with heat and nitric acid. The alkali dissolves the bacterial proteins and the filtrate gives an albumin test even when there was none in the urine when passed. This accounts for the high percentage of albuminuria reported from some of these central laboratories.

The method is to be condemned. It seems difficult for some medical men to get away from the idea that bacteria are vegetable organisms. They consist mostly of protein, soluble in strong alkali, especially on the application of heat. The test, applied in the way mentioned above, gives no reliable information and is misleading. It has been suggested that the urine containing bacteria should be passed through a Berkefeld filter and the heat and nitric acid tests applied to the filtrate. This is open to two sources of error. In the first place, albumin when present may be held in the filter and thus escape detection. In the second place, some of the bacterial proteins may be in solution without the addition of alkali and may pass through the filter.

—V. C. W.

PREPARATION of Protein Poison from Egg White.—Vaughan and Wheeler prepare the protein poison from egg white in the following manner: The whites of two dozen or more fresh eggs are dropped, with frequent stirring, into two or more liters of 96 per cent alcohol. After 24 hours the supernatant fluid is decanted and replaced by absolute alcohol. After another day, during which the coagulum is frequently stirred, it is drained into a filter, put in hulls and extracted in Soxhlet's for four days with absolute alcohol and for the three following days with ether. Thorough extraction with these reagents is necessary in order to remove all traces of saponifying substances. The contents of the hulls are rubbed into a fine powder, first in porcelain, and then in agate mortars. In this way a snow white powder of egg protein is obtained, and may be kept indefinitely in a wide-mouth bottle.

One liter or more of a 2 per cent solution of caustic soda in absolute alcohol is prepared. Care and patience are needed in this preparation. Purified sodium hydrate is needed. This should be quickly weighed, since it is deliquescent and rapidly absorbs water from the air. The broken sticks of alkali are gotten under absolute alcohol in a large porcelain mortar and rubbed into solu-

tion. This solution is diluted with absolute alcohol so as to make a 2 per cent solution.

A definite amount of the powdered egg-white is weighed out and placed in a Florence flask from which all trace of moisture has been removed by rinsing with absolute alcohol. The dry powder in this flask is covered with twenty times its weight of the 2 per cent solution of alkali. The flask is attached to a reflux condenser and to a mechanical stirrer. (The stirrer is not absolutely essential, but when not used the flask must be constantly shaken by hand.) The flask is heated in a water or steam bath for one hour, care being taken that the temperature of the contents does not rise about 78 degrees C. At the expiration of one hour the contents of the flask are allowed to cool and subside and the supernatant fluid is decanted. Three extractions of the insoluble part, with the 2 per cent alkaline solution, are necessary to complete the change of the protein into poisonous and non-poisonous fractions. The former is soluble in the alcoholic solution, while the latter is insoluble.

The combined alcoholic extracts are carefully neutralized with hydrochloric acid, which precipitates the alkaline base as sodium chloride. This is removed by filtration and the filtrate evaporated, better in vacuo. This leaves the crude poison, the action of which can be demonstrated on guinea-pigs, best by intraperitoneal, intravenous or intracardiac injection. The poison has not been obtained in a pure state and its chemical structure remains unknown. It may be partially purified from the crude product obtained as above by repeated solution in absolute alcohol and evaporation and still further by precipitation from alcoholic solution by alcoholic solution of platinum, mercuric or cupric chlorides, and the removal of the base with hydrogen sulphide. In the purest form in which it has been obtained it kills guinea-pigs with the symptoms and post-mortem appearance of anaphylactic shock in dose of one-half milligram on intravenous or intracardiac injections.

—V. C. V.

THE Preparation of Specific Precipitins.—In 1897 Kraus found that the serum of an animal which had been repeatedly treated with a culture of a given bacillus gives a precipitate when added to the filtrate of the same organism. It was soon found that this reaction is specific. Cholera serum precipitates only cholera filtrates; typhoid only typhoid filtrates, etc. Later, Tschistowitsch and Bordet demonstrated that this is a general protein reaction. A rabbit which has received small injections of horse serum furnishes a serum which precipitates horse serum in high dilution and no other proteins in like dilution. One treated with cow's milk supplies a serum which precipitates cow's milk and no other in the same dilution. One treated with egg-white precipitates the proteins of this mixture and no other in the same dilution. Practically this test is now employed in the identification of blood stains in medico-legal investigations in the detection of mixed meats and in the study of the blood relationship of species and genera. If a blood stain be dissolved in physiologic salt solution, filtered and mixed with the serum of a rabbit which has been repeatedly treated with human blood, a precipitate will form if the stain be human blood. If a hamburger steak be extracted with water the clear filtrate

will be precipitated with the blood of a rabbit which has been treated with horse serum, provided the steak contains horse meat. If the steak contains both horse flesh and beef, the extract will give precipitates with an animal treated with horse serum, also with that of one treated with ox serum. In this way the flesh of any animal, the cat, dog, etc., can be detected in the steak. This is frequently employed in municipal laboratories in Europe. The serum of a rabbit previously treated with human serum will precipitate this serum in high dilutions; that of the higher apes in less dilution and will fail to precipitate the sera of the lower apes and of all other animals. This supplies confirmation of the teaching of zoologists concerning the blood relationship of animals. The best animal for the supply of specific precipitins is the rabbit. It should receive at intervals of from three to five days intraperitoneally or subcutaneously from 3 to 5 c.c. of the selected protein and ten days after the last injection the serum of the animal is in condition to give the specific precipitin test. The substance injected into the rabbit is known as the precipitinogen (producer of the precipitin). The specific substance in the serum is known as the precipitin, and the product is designated as the precipitate.

—I. C. I.

AGGLUTINATION.—In 1889 Charin and Roger, in studying the action of the serum of sick and immunized animals on homologous bacteria, observed that the *Bacillus pyocyaneus* behaves peculiarly when placed in the serum of an animal previously treated with this organism. In the serum of a normal rabbit this bacillus grows as it does in bouillon forming an opaque culture, while in the serum of an animal previously treated with this bacillus, it forms floc-cules which soon subside, leaving a clear, supernatant fluid. Two years later Metchnikoff observed similar behavior in the vibrio which bears his name. He wrote, "In the blood and serum of normal vaccinated guinea-pigs, the vibrio develops as it does in the ordinary liquid media, the individual organism retaining their motility and remaining distinct from one another. On the other hand, in the blood and serum of vaccinated animals the vibrios become im-mobile, and form smaller or larger floccules which float in the fluid."

In 1893 Issaeff, and later he and Ivanoff, observed the same phenomenon and described it as follows: "In the serum of non-immunized guinea-pigs the vibrio develops rapidly and after from four to five hours at 37 degrees, there is a uniform cloudiness throughout the fluid, while the surface is covered with a scum; but in immune serum, the microbes sink to the bottom of the tube, while the supernatant fluid remains clear. This condition continues for from eight to nine days and it is not until the tenth day that the solution becomes cloudy and a scum appears on the surface."

In 1896 Gruber and Durham demonstrated that this reaction is specific and pointed out its value in the identification of bacteria. A given bacterium is the typhoid bacillus if it is agglutinated or clumped by the serum of an animal immunized to the typhoid bacillus. This use of the phenomenon of agglutination is the only one suggested by Gruber and Durham, and it has been overshadowed by the more practical application discovered by Widal, who pointed out that it may be employed in the diagnosis of disease. If the serum of one

sick with a fever agglutinates the typhoid bacillus in high dilution the disease is typhoid fever.

The period in the progress of typhoid fever when marked agglutination is in evidence varies widely. As a rule it is not before the seventh day, but it may occur as early as the second, and it may be delayed until the second week and even later. Likewise the disappearance of the reaction after recovery is variable. It may fail ten days after the disappearance of the fever, and it may continue for years. In intensity the reaction is variable and bears no recognizable relation to the severity of the disease. The method of applying the test, as recommended by Widal and Sicard is as follows: "A number of small diameter tubes, each containing 1, 2, 3, 4, and 5 c.c. of bouillon are kept on hand. When a test is to be made, one adds a drop of the serum to each of these tubes and then inoculates each with a typhoid culture. The tubes are then placed in the incubator for from four to six hours. At the expiration of this time it may be seen at a glance in which tubes agglutination has taken place, since these will be unclouded and the floccules will be seen at the bottom. The first tube is a dilution of 1:20, the second 1:40, etc."

Johnston showed that a drop of blood allowed to dry on non-absorbent paper may be transported any distance and kept indefinitely and still be capable of giving the agglutination test after solution in water, but this does not permit accuracy in determining the dilution. Agglutination may be observed under the microscope when only one drop of blood is available. When serum is sent to a distant laboratory for examination formaldehyde may be added, but a preservative is not necessary, since putrefaction does not interfere with the test. A positive diagnosis should not be made unless marked agglutination is observed in a dilution of not less than 1:50, and even in this dilution there is chance for mistake. In experimental animals sera can be obtained which will agglutinate typhoid bacilli in dilutions as high as 1:1,000,000 and colon bacilli 1:2,000,000.

In the identification of bacteria agglutination should take place in high dilutions, which, however, vary with the organism. The strongest proof that Shiga's bacilli is a cause of dysentery lies in the fact that its cultures are agglutinated with high dilutions of those ill with dysentery.

—V. C. V.

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EDITORIALS

The New Journal

WHY start a new medical journal? This question will arise in the minds of all who see this publication. It is a perfectly proper question and one which we have asked ourselves many times in the past few months. It does seem that the medical profession is already oppressed by a great flood of literature, some good, some bad, and much of it indifferent. There are general and special organs, weekly, monthly and quarterly, in all the languages of the civilized world. Then why start another? Why ask the medical man to add another to the great heap of periodicals that—often unopened—burden his table? To these questions we have given serious consideration and finally have decided that a medical journal, of the kind which we hope to make this, will be of value to the profession. At present there is a wide chasm between the research man and the practitioner. The former mines the ore, the latter refines it and shapes it into useful implements. The work of the one supplements that of the other. The world is not benefited nor medicine advanced until the work of both has been done. Modern medicine consists of those facts gathered from the various sciences which may be utilized in the prevention or relief of disease. Scientific discovery must precede practical application, but until the proper use is found the value of the discovery is wholly potential. The basic aim of this journal will be to bring discovery and its application closer together, to supply the research man with a strictly scientific organ through which he can report the results of his labors, and to suggest to the practitioner how he may use the latest discoveries. The journal will be filled with original

articles and editorial reviews. In the former we hope to present much of the best productive research done in this country. In the latter there will be a record of the advances in the physical, chemical, and biological sciences, so far as they have medical significance, made by experts. Contributions to the physiology, nutrition and life phases of man, discoveries in etiology, pathology, and the development of disease processes, improved methods of diagnosis and treatment will be reported, and the endeavor will be to keep the practitioner close upon the heels of the discoverer. The man who relies upon text-books to keep up with his profession today is falling far behind in the race. Special scientific periodicals are numerous and of these our own country is now producing some of the best, but the practitioner can hardly be expected to read any large number of these and there is certain benefit to the profession in having much of the work reported in these special organs gleaned and made readily comprehensible. Mere abstracts without proper interpretation are of but little value. We propose to review original work especially that which will be of either intellectual or practical value to the practitioner. These reviews will be accompanied by comments and possibly by criticism from the reviewer. Abstracts of scientific contributions by those not well informed as to what others have done along the same line are of but little value. The editorial staff has been selected with the intention of having an expert present with proper estimate the results of the latest scientific investigations. Only those expertly trained can properly interpret work of this kind.

The man who attempts to practice medicine without laboratory aid belongs to a past generation, and fails to do justice to his patients or credit to himself. Our purpose will be to present the work of our best investigators to the practitioners, either directly in the form of original contributions or as reviews of work published elsewhere. One of the most encouraging evidences of the scientific advance of medical practice in this country is the increasing appreciation and utilization of laboratory methods in diagnosis. Without proper diagnosis treatment fails and in a large per cent of the cases coming to the practitioner the former is quite impossible without the aid of the laboratory. Our endeavor will not be limited to curative medicine, but will include much in the art of the prevention of disease.

The ultimate goal of science is the domination of the forces of nature and their utilization in the betterment of mankind. In this great work the medical profession must play an important part and it is our one desire that this journal may aid in carrying on this work.

—V. C. V.

Phagocytosis

IT has been assumed for many years that phagocytosis is an active process, and that it is associated with the power of active motion in certain cells. These cells send out projections from their bodies toward certain particles which happen to be present in their environment and presently surround them, and in certain instances destroy them. In the process of phagocytosis, chemical reactions have always been given a prominent place, for it has been held that certain variations in chemical reactions in some way account for the move-

ments of cells (chemotaxis). A recent communication by Oliver¹ discusses the crenation and flagellation of erythrocytes and throws some light upon the process which is responsible for amoeboid activity of cells. Crenation is apparently the opposite of flagellation and can be produced entirely by physical means. For instance, Oliver shows that if one takes a crenated blood corpuscle and merely touches it with a fine glass needle, which is immediately withdrawn, the cell becomes round again and appears optically normal, and remains so. If, on the other hand, a glass needle is brought close to a normal red corpuscle, but not touching it, it immediately crenates, and the degree of crenation depends upon the proximity of the needle to the cell. By following the physical method of Oliver, cells may be crenated and uncrenated at will and more or less indefinitely. Since the phenomenon of crenation has always been attributed to hypotonicity of the surrounding fluid, it is interesting to note that the phenomenon can be produced regardless of the tonicities—that it occurs equally well in isotonic, hypotonic, or hypertonic solutions. The phenomenon of crenation seems therefore to depend upon conditions of surface tension which are produced in making the ordinary mounts of fresh blood, and it illustrates the extreme irritability of cellular protoplasm. Oliver continues with some observations on flagellation which were suggested to him by Kite's work. He noticed that soon after making fresh preparation of blood, many of the cells were crenated, and that some 40 or 50 minutes later the cells were seen to possess long processes, some motile and some non-motile. By means of such motile process the cells are moved across the field of the microscope. These processes may become free from the cells and show independent motion. Non-motile processes may be made to beat by merely touching them with a fine needle held in the Barber holder.

These observations lead very directly to those of Kite and Wherry² upon the mechanism of phagocytosis. They suggest that phagocytosis depends upon the physical conditions of the surface of the phagocytic cells and that the engulfing of particles is a purely passive process which depends upon protoplasmic streaming within the cells. They believe that such substances as opsonins act in increasing phagocytosis merely because they increase the "stickiness" of the cells, and that phagocytosis depends essentially upon the relative stickiness of phagocytes and bacteria. Their experiments indicate that the physical manipulations invoked in making opsonic preparations are sufficient to account for the part of the phenomenon of phagocytosis in such preparations, and that bacteria stick to the phagocytes best in the presence of unheated serum because of the presence of something in the serum which makes them more sticky. Incubation of specimens following the Wright technic for determining the opsoninic index seems, from these experiments, to be valueless, for the phenomenon will occur at any temperature from 11 degrees C to 37 degrees C. The process of mixing is important, for a very careful, quiet, technic reduces phagocytosis.

A recent report by Lawson³ adds emphasis to the work of Oliver, and Kite and Wherry. Miss Lawson shows that contrary to the current conception, the malarial parasite is not intracellular, but extracellular and perhaps pericellular. They become attached to the erythrocytes at certain points which are referred

¹Science, 1914 (40) 645.

²Journ. Infect. Dis., 1915 (16) 109.

³Journ. Exp. Med., 1915 (21) 584.

to as "mounds" which the parasites tend to surround with pseudopods. Here again is evidence that mechanical contact produces changes in the contour of the red cells and that at the same time the relative stickiness of the protoplasm of cells and parasites is increased so that they adhere to one another.

Upon the basis of these researches which indicate that purely physical forces are of first importance in explanation of phagocytosis, the older work upon which our current accepted conceptions are based, must be repeated, and interpreted from a new angle.

—P. G. W.

*Recent Work on Blood Pressure Measurements in Man**

IT would seem a comparatively simple problem to ascertain, by laboratory experiments, the actual pressures in the blood streams at which changes, that are similar to those observed in making blood pressure measurements in the clinic, occur in the behavior of the pulse, when varying outside pressures are applied to the artery. Connecting a mercury manometer with a branch of the artery on which the "clinical" methods are being tested, and then comparing the readings taken by the two methods would appear to be all that is necessary in order to test the reliability of the clinical method. But unfortunately the information thus obtained has been of only limited value, because the dissimilar anatomical relationships of the vessels in laboratory animals and in man introduce physical differences which alter very considerably the behavior of the pulse. In view of these facts, and because of the enormous practical importance to the clinical observer of knowing just exactly how reliable are the methods at his disposal for gauging the blood pressure, two eminent physiologists, Professor J. A. MacWilliam, of Aberdeen, Scotland, and Professor Leonard Hill, F.R.S., of London, along with several collaborators, have been, and are at present, engaged in throwing further light on the question. In the following review we shall summarize the main findings of these workers, in so far as they would seem to be of value to the clinical worker.

There are three aspects of the arterial blood pressures which it is important to measure, the *systolic pressure*, the *diastolic pressure*, and the *pressure pulse*. The first means the high pressure attained in the blood stream during each heart beat, the second, the lowest pressure occurring between the heart beats, and the third, the difference between the systolic and the diastolic pressure. A fourth value—the *mean blood pressure*—is the average between systolic and diastolic pressures. Each of these is given in terms of the height in millimeters of a column of mercury, and in the brachial artery of a healthy young man while lying down they usually amount to 115 m.m., 85 m.m., and 30 m.m., respectively.

Before proceeding to review the actual methods of measurement it will be well to consider the *practical value of the results in elucidating the actual conditions in the circulation*.

(1) *Systolic Pressure*: Even when the systolic pressure is accurately measured—and we shall see shortly that some care has to be exercised in do-

*For convenience this editorial has been divided into two parts, the second installment to appear in the November issue. The literature is given at the end and is referred to in the context by numbers.

ing this—"it gives very insufficient evidence as to what is going on in the circulation," for all it tells us is the momentary highest pressure transmitted to the blood column by each heart beat.⁵ Thus, in heart block the systolic pressure may be very high and yet the mass movement* of the blood quite defective, as evidenced by symptoms of cerebral anæmia; or again, in cases of slow cardiac failure in which cyanosis, dyspnœa, etc., are pronounced, the systolic pressure may be about the normal level.

(2) *Diastolic Pressure:* Measurement of the diastolic pressure is important not only because it gives us the load which the vessels and the aortic valves must constantly bear and the resistance to the opening of these valves at the beginning of systole, but because it helps us to gauge the peripheral resistance. In using the diastolic pressure for this purpose, however, it must be remembered that the heart rate also affects it, a slow beat making it fall and a quick beat making it rise. It is therefore possible to have a normal diastolic pressure, either with a low peripheral resistance and a quick pulse, or with a high peripheral resistance and a slow pulse. In these cases the arterial walls may be perfectly healthy. When the walls of the arteries are stiffened, the diastolic pressure becomes lower (if they were perfectly rigid the diastolic pressure would be zero). In such cases, however, the pressure pulse will be very marked. A high diastolic pressure in cases with stiffened arteries, and no quickening of the pulse, is very significant of a high peripheral resistance. It is of great practical importance to remember that a constantly high diastolic pressure entails a much greater strain on the vessel walls than the transient systolic pressure, and should be taken as a danger signal of possible rupture.

(3) *Both Systolic and Diastolic Pressures—Pressure Pulse:* Can we assume that with normal systolic and diastolic pressures, and a normal heart rate, the circulation is normal? In the vast majority of cases we can, but not always. Thus, in cases where the aortic orifice is narrowed, so that less blood is discharged during systole into the vessels, we may at the same time have a narrowing of peripheral blood vessels, thus increasing the resistance to outflow and compensating for the deficient cardiac output, so that the above pressure readings are normal. But obviously, under these conditions the mass movement of the blood must be deficient, as is frequently evidenced by the general symptoms of dyspnœa, pallor, cerebral anæmia, etc., so common in aortic disease.

These facts will make it plain that although the estimation of *both* systolic and diastolic pressures is of very great importance and value in clinical diagnosis, yet "they must on no account be regarded as superseding or rendering unnecessary the use of other sources of information regarding the condition of the circulation. . . . Error from misuse of the results of blood pressure estimation, by blind adherence to mechanical standards, is easily avoided by common-sense application of other obvious signs taken in correlation with the blood pressure data." (MacWilliam.⁵)

The Measurement of the Systolic Pressure: Auditory, visual and tactile methods exist for the measurement of the systolic pressure. In all of them an armlet is placed around the arm or leg and the behavior of the pulse is exam-

*By mass movement of blood is meant the volume of blood which passes a given point in the circulation in a given time. Volume flow means the same thing.

ined, by one or other of the above methods, lower down when the air pressure in the armlet (which should be 12 c.m. broad) is raised and lowered by means of a suitable pump. In the *auditory method*, a stethoscope or phonendoscope is placed immediately below the armlet. When the pressure in the armlet is raised above the systolic, no sound is heard, but on gradually decompressing, a distinct tapping sound becomes audible with each heart beat. This indicates the moment at which blood begins to force its way, during systole, along the vessel beneath the armlet, and the pressure now registered by a manometer connected with the armlet is taken as the systolic (systolic index). We shall explain immediately how, on further lowering the armlet pressure, the diastolic pressure is indicated.

The *tactile method* consists in palpating the radial, or dorsalis pedis, or posterior tibial arteries. No pulse is palpable until the armlet pressure has been lowered to a few millimetres below the auditory systolic index. This tactile systolic index is, therefore, always below the auditory; if it is not so, it indicates that some error has been made in the application of the apparatus, most usually in the phonendoscope. If readjustment does not bring the two "indices" into proper relationship, the auscultatory method cannot be relied upon, either for systolic or diastolic readings (MacWilliam⁵).

The *visual method* consists in watching the pulsations in the manometer connected with the armlet. With pressures above systolic very faint, if any, pulsations are visible—this depends on the delicacy of the manometer—but when the pressure is lowered to the systolic, the pulsations become more pronounced.

Of course, each of these methods can be employed while the armlet pressure is being gradually raised instead of lowered, but the readings are often different and we shall have to consider later the reasons for this.

Meanwhile there are several *conditions affecting the gauging of the systolic pressure* which we shall have to consider.

A. The Mode of Compression of the Artery: Leonard Hill and his collaborators⁶ have found that there is an extraordinary difference in the amount of pressure required to obliterate an artery, so that no recognizable pulse occurs below, according to whether the vessel is compressed with or without at the same time compressing the tissues in which it lies. This has been shown both clinically and experimentally. Thus, when the small bag of the pocket sphygmometer (Leonard Hill's) was placed on the radial artery, embedded in the tissues of the forearm, a systolic pressure of 110 m.m. Hg. was observed, but when applied a little lower down to an aberrant radial, the pressure was only 35 m.m. Hg. Similar differences can be demonstrated in the leg vessels and the dorsalis pedis. Experimental corroboration of these clinical findings was obtained by exposing an artery in a living animal: when this was compressed in a tube full of saline, the pulse, distal to the compression, was not obliterated until the pressure was raised just above the systolic pressure of the blood in the artery, whereas when the same artery, placed on bone, wood or glass, was compressed locally, as by arranging an armlet so that it did not embrace other tissues, the pulse was abolished by pressures that were not only considerably under systolic but even under the diastolic pressure. For accurate

systolic readings it is therefore very important to use an armlet which is at least 13 c.m. wide.

B. Discrepancies between Systolic Readings on Compression and Decompression of the Armlet: The pulse below the armlet may reappear on decompression at a lower pressure than it disappears on compression. This discrepancy is most marked when the decompression is done quickly. By means of a circulation model, the general construction of which will be described later, Hill and his collaborators⁷ have succeeded in demonstrating that the above discrepancy is due to the fact that the full force of the pulse does not reach the forearm until all the vessels of the upper arm have become filled with blood and therefore tense. This explanation was further corroborated by finding that it took much longer than usual for the pulse to return at the wrist on decompression when the forearm had been emptied of blood, by elevating it and applying a bandage before compression and then removing it just before decompression.

C. Discrepancies in Systolic Readings from Different Limbs: It is not uncommon to find that the systolic pressure in the leg is higher than that in the arm, even when the patient is in the horizontal position. It is particularly in cases of *aortic regurgitation* that the discrepancy is seen. Hill, McQueen and Flack⁶ have made extensive investigation of the causes for this difference and conclude that it is partly due to: (1) The greater lumen of the aorta, iliac and femoral arteries in comparison with that of the subclavian and brachial; the wider the lumen, the greater the amplitude of the pulse wave. (2) The pulsating support which the relatively massive abdominal organs and the tissues of the thigh give to the main arteries prevents the damping of the crest of the pulse waves. (3) The leg arteries are probably held in a more supported state, and are less labile than the arm arteries, so that the pulse is conducted to the leg with less diminution in force. As an outcome of experiments with artificial models, already referred to,⁵ these same authors place emphasis on the last of the above explanations; to quote, "The explanation of the difference which pertains between the arm and leg systolic readings taken in the horizontal posture, in cases of aortic regurgitation, is to be sought in the difference of the lability of the artery and the condition of the peripheral circulation."

Similar discrepancies in systolic readings between different limbs are also observed, especially on the first estimation of the pressure, in cases with *thickened arteries*. MacWilliam and Kesson² have made an extensive investigation of the observed pressures in such cases, and have found that the discrepancy becomes greatly reduced on continued or repeated compression of the artery, and that the fallacious reading (i. e., the one that is high) is decidedly less when the pressure of reappearance rather than that of disappearance of the pulse is measured. It is pointed out, however, that the repeated compression of the hardened vessel may introduce a fresh complication in the gauging of the pressure, on account of the rise in general blood pressure. By investigating the behavior of fresh surviving arteries in different conditions of contraction and relaxation in a circulation schema (see below), it was found that repeated compression caused the contracted artery to behave, qualitatively at least, like the normal artery. The extraordinary degree of change in pressure sometimes ob-

served in the artery embedded in the human limb after continuous pressure could never be duplicated in excised arteries unless these were extremely contracted (such as pathological arteries from the legs of horses, or arteries artificially contracted by barium chloride), arteries, that is to say, which are very much more contracted than is ever possible in the human limb. The explanation offered by MacWilliam, etc.,⁵ for this peculiar behavior of the arteries is along the lines suggested long ago by von Recklinghausen, namely, that the hypertonic artery when compressed externally does not, as does a normal vessel, become completely closed, but its walls become apposed at the middle part while chinks still remain at the sides. The blood continues to pass by these chinks, and to obliterate them a very considerably higher arterial pressure is required. By continued or repeated compression it could be shown, in excised hypertonic arteries, that the tonic contraction was temporarily removed so that the walls of artery become apposed in normal fashion. If this is the true explanation of the different systolic pressures often observable between the arm and leg arteries, it should follow that, although the pulse does not disappear in the leg at as low a pressure as in the arm, there would be some demonstrable change in its character at the pressure at which it disappears from the arm. Such was found to be the case in a patient showing an obliteration value (i. e., systolic index) of 115 for the upper arm and 198 for the leg; "at 116 the pulse in the leg though not obliterated was very notably cut down in volume; it persisted as a pulse of small volume with very little alteration till it became obliterated. . . . Repeated compression was tried and was found to give marked lowering in the leg readings at successive tests, namely, 198, 186, 168, 149, 148 m.m." During all this time the arm pressure remained steady at 115 m.m. The differences were much more marked when the calf vessels were used than with those of the thigh.

Besides these abnormal cases of aortic disease and contracted arteries, we may find even with *normal vessels* that the systolic measurements, taken at different parts of the circulation, do not agree; more especially after muscular exercise. Thus, Hill, etc.,⁷ state that the systolic reading in the thigh is considerably above that in the calf in normal boys, such readings as the following being reported in these different cases:

Thigh	Calf
165 m.m. Hg.	135 m.m. Hg.
155 " "	120 " "
105 " "	80 " "

In other cases (designated as patients, however, see footnote on p. 510, *loc. cit.*) the readings were considerably higher in the calf than in the thigh, and the differences could not be accounted for as due to protection of the artery from compression by fasciæ, etc.

D. Increase in Pulse Beat at Wrist at the Beginning of the Rise in Armlet Pressure: When an armlet is placed on the upper arm and the pulse is felt at the wrist, the latter will often increase perceptibly in force as the armlet pressure is raised from zero to 50 m.m. Hg.⁷ In some persons this, seemingly paradoxical, behavior is very pronounced; thus, in a patient whose systolic pressure was 130, and diastolic 80 m.m. Hg., the increase in force of the

pulse became perceptible when the armlet pressure was raised to 36-40 m.m. Hg., but when the same patient did muscular exercise, and so caused the difference between the systolic and diastolic pressures to become greater (i. e., increase of pressure pulse), the increase in the pulse was observed with 10 m.m. of pressure in the armlet. In aortic disease also, where there is a similarly large pressure pulse, even during rest, the augmentation on the radial pulse was apparent with 5-10 m.m. armlet pressure. Similar augmentation of the pulse in the calf was obtained during increase in the pressure of an armlet placed on the thigh.

Not only can the augmentation of the pulse be felt by the finger, but it may be demonstrated by using a sphygmograph. It is also associated with the appearance of a distinct sound heard by listening over the artery with a stethoscope or phonendoscope.

When we come to describe the method by which the changes in pulse sound may be used for measuring the systolic and diastolic pressures (see below), we shall see that, starting with a high armlet pressure and gradually lowering it, no sound is heard until some blood succeeds in passing the armlet during each systole. As the pressure is lowered, this sound gets louder, until at a certain armlet pressure, which corresponds to the diastolic, it suddenly gets less. At lower pressures it still persists for a time, and the pressure of its final disappearance is that at which the described augmentation of the pulse is perceptible. Thus, in the patient already referred to above, there was no sound discernible at the elbow until the armlet pressure had reached 30-40 m.m. Hg., although after muscular exercise it became evident at 10 m.m. In entire conformity with these findings is the well-known clinical experience that in case of aortic disease a pulse sound is heard over the arteries without any armlet pressure at all. This sound becomes more marked with slight armlet pressures.

The cause of the sounds is ascribed by Hill, etc., "to the impact of the systolic wave vibrating the tense artery and its branches. . . . This sets the mass of tissue enclosed in the armlet into vibration, the vibrations becoming audible to the ear as sounds. . . . Therefore, the sound is dependent on the pulse-pressure range of the blood stream which enters the armlet. . . . If the pressure within the armlet is raised, the systolic wave is reinforced by the increased tension of the arteries under the armlet, and the sound becomes louder."⁸

A very interesting simple experiment corroborating this explanation of the sound (viz., that it is due to the sudden tension produced by each systole on vascular tissue under the cuff), consists in placing two armlets around the arm with a phonendoscope between them. When the pressure in the upper armlet has been raised sufficiently to make the sound audible, inflation of the lower armlet to a pressure above the systolic does not cause the sound to disappear, but rather to increase. Complete stoppage of the circulation does not, therefore, diminish the sound, thus indicating that it must be due to tension changes in the vessels.

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—J. J. R. M.

The Constituents of Diets—A Review

OSLER has said that the physician who wishes to obtain a sound knowledge of the natural history of disease, must adopt Morgagni's method of "anatomic thinking." It is becoming more and more evident that the modern physician must go beyond this and adopt the method of physiologic thinking, and he should remember that this implies physical and chemical thinking. The medicine of the present is based more largely than ever before upon the fundamental sciences, physics and chemistry and the evidences are that these sciences will play an ever increasing role. The "method of Zadig" (or of Morgagni) is the observational part, but under this, furnishing the foundation for logical diagnosis, prognosis, and treatment, is pure chemistry.

Nowhere in the literature is this point of view more clearly illustrated than in the reports of investigators dealing with the influences of diets. These reports form two general groups, those which, like the ones of Schaumann, Funk and others, deal with certain diseases like beriberi and scurvy; and the ones of Osborne and Mendel, and others, which have to do with the effects of dietary re-arrangements and limitations in experimental animals, and the relationships of the items in the diets to growth.

There are, of course, two sides to the question of diets. The state of nutrition of an individual is dependent upon certain conditions in the external world, such as the quality and quantity of nutritive material which is available, and also upon the internal structure of the body upon which is dependent the powers of the individual to make use of food. It makes no difference how much food nor how good food is available to an individual if his tissues are not able to assimilate it, nor does it matter how *well* the body is, if the food supply is not sufficient in quantity and quality,—he starves just the same. In one case he may not grow,—which is partial starvation.

It may be trite to call attention to the fact that the amount of food that is necessary for an individual, depends upon his age, the amount of muscular

activity he displays, the climate (or the weather), and certain other factors such as the activity of one or more of the ductless glands, as well as indefinite and not-understood ones which we group together as "inherited." Everyone realizes the atrociously large appetites of children who are active, but one sometimes forgets that the dosage of milk which is accepted for a six-months' child is for the average hospital child and not (necessarily) for the healthy, active child that would grow faster if it had more food. One knows that what is needed in hot weather is food enough, and plenty of water to counteract the heat produced in burning the food. The value of peppers in the tropics is that (given enough water) perspiration is stimulated and the result is cooling.

If the normal amount of food is consumed and if the food is used by a normal body, physiologic growth will occur. But if too much food is taken, or if the body is inactive, food will be stored in the form of fat. Lack of exercise is the great cause of obesity. That is why, in part at least, the contented person tends to stoutness, for other things being equal, the contented person "rests" more; his mind and muscles are oftener at ease and so he burns less of his food. "Laugh and grow fat" is a physiologic truth. But many adipose persons are not contented, and many are very active. In such cases one of two things is apt to be wrong,—either they have inherited a type of metabolism which is not normal for the race, or something is wrong with his organs of internal secretion—especially the pituitary, the thyroid, or the sex glands. Similarly the thin individual is one who eats too little, or exercises too much, or is nervous; who has some ductless gland disorder, or who is "just naturally skinny."

But even so, we have looked at but part of the picture. We have taken for granted that the food is good in *quality*. An individual must have, not only good food, but the composition of the food must be proper. The diet must contain a certain proportion of protein, of fats, of carbohydrates, and of salts, beside the right amount of water.

It has been known for a long time, that if enough protein is not eaten the body suffers, and something like the same thing has been known of the other dietary constituents. The seafaring people learned that salt meat and bread would not keep a man alive and well, even though it contained the theoretically proper food values. Such a diet produces scurvy. If mere boiling of milk for babies reduces its value as a food, it is not because the *food values* are not there, but because certain constituents are physically changed.¹ Frank² has offered evidence, for instance, that the digestibility of egg-white is influenced by the temperature at which it is coagulated. A diet of certain kinds of rice, which are lacking in phosphorus and perhaps other substances produces beriberi in the tropics; and a diet of dried grain (oats) and water produces a disease like rickets or scurvy in guinea-pigs. A little fresh food, *living food*, such as salad, or fresh milk, or even merely milk whey, will correct such diseased conditions. It is probable that pellagra is one of the diseases caused by unbalanced or deficient food. It is an interesting fact that the substances which will correct some dietary defects are active in such small amounts that they

¹Compare with Rupp: Bull. 166, Bureau of Animal Industry

²Journal of Biolog. Chem., 1911 (9) 463.

can scarcely be detected. They are called collectively for the present the "vitamines." Funk³ has suggested that these substances have a certain relation to the activities of the glands of internal secretions.

Since a balanced diet must be composed of more or less certain amounts of protein, fat, carbohydrate, salts and water, it is obvious that an inadequate diet may be deficient in one of several ways,—it may lack any one of the necessary constituents, or it may lack more than one. It is interesting that we have come to know, of recent years, that the lack may be not merely a gross one, as exemplified in absence of proteins, but it may be a chemical one and dependent upon the structure of the protein. Perhaps much the same state of affairs exists in regard to the fats and carbohydrates, perhaps also to the salts.

A great deal of our newer information concerning the proteins we owe to Osborne and Mendel.⁴ They have experimented with pure proteins and have shown definitely that certain amino acids are indispensable in food. With certain proteins, lacking certain amino acids, maintenance, but not growth, occurs. With other proteins lacking certain other amino acids, not even equilibrium can be held. For instance, they have shown that the protein of corn (zein) is an inefficient protein, and that when zein serves as the sole source of nitrogen in a diet nutritive failure results. It is a perfectly digestible protein but lacks tryptophan and lysin. The addition of both these latter to zein completes it. Add tryptophan alone and equilibrium is maintained; add both, and growth follows. Gelatin is not an efficient protein because it lacks tryptophan. Also they have shown that the protein of wheat (gliadin) which will serve in maintaining equilibrium, will not permit of growth, but add lysin to gliadin, and growth results. Gliadin is evidently a better source of nitrogen than zein. Lysin is present in many foods, for instance, 8 per cent in lactalbumin; 7.59 per cent in ox muscle; 4.81 per cent in egg yolk; 4.98 per cent in peas; and 4.58 per cent in beans; 7.61 per cent in casein. The addition of any of these to a corn or wheat dietary (in which connection one thinks of breakfast foods) adds to its efficiency, and tends to insure growth. These facts apply to adult animals to be sure, but they apply more trenchantly to young animals.

Osborne and Mendel have also added much to our knowledge of the food value of fats.⁵ They found that young animals (rats) did not complete their growth upon a diet of isolated proteins, starch, protein-free milk and commercial lard or olive oil, but if butter fat replaced the lard, growth went on. They also found that cod liver oil could be substituted for the lard. McCollum and Davis,⁶ and Osborne and Mendel have also found that if egg fat is added to a lard containing dietary, growth will occur. Evidently the type of fat in a diet is important also.⁷

Little is said in the literature regarding the influence of carbohydrates.

³Journ. Physiol., 1914 (47) 475.

⁴Osborne and Mendel: J. Biol. Chem., 1914 (17) 325; 1914 (18) 1; Med. Record, 1914 (85) 737; cf. also Jour. A. M. A., 1914 (63) 190.

Mendel: Jour. A. M. A., 1914 (63) 819.

⁵Osborne and Mendel: J. Biol. Chem., 1914 (17) 325; *ibid.*, 1914 (18) 1; cf. also J. A. M. A., 1914 (63) 190; Med. Record, 1914 (85) 737.

Osborne and Mendel: J. Biol. Chem., 1913 (16) 423; *Ibid.*, 1914 (17) 401; *ibid.*, 1912 (12) 81.

⁶McCollum and Davis: J. Biol. Chem., 1913 (15) 167.

⁷Mendel: Jour. A. M. A., 1914 (63) 819.

Evidently their chief function is to furnish the sources of heat for the body. They do not form an indispensable part of the dietary so far as growth is concerned. They seem to be more or less alike. Any of the common carbohydrates may be efficient. The usual one used in experimental diets is starch. It appears, however, from the recent experiments of Tachau⁸ that an addition of sugar to an already adequate diet may result disastrously—how, one cannot say. Tachau obtained similar results with certain salts, and fats. It is possible that the easily burned carbohydrates may, under certain conditions, furnish too much heat; then, unless the windows are opened, the house may get too warm, or they may, in too large quantities be incompletely burned and produce substances which interfere with protoplasm (cellular) chemical processes (cellular asphyxia). The split products of both carbohydrates and fats may indeed menace the body, and lack or over-abundance of salts may surely change the reactions of the living cells. In interpreting the results of experiments, one may bear in mind that the “living organism may be regarded as a highly unstable chemical system which tends to increase itself continuously under the average of the conditions to which it is subject, but undergoes disintegration as a result of any variation from this average.”⁹ There seems to be no evidence that in the so-called *Mehlnährschaden* the starch itself is the harmful agent. It is only when given in excessive amounts for long periods that harm results, and this is due not so much to the starch as to the attendant deprivation of other food stuffs.¹⁰

If an animal is fed upon a diet consisting of the proper amounts of protein, fats, carbohydrates, and water, with no salts, it will die more quickly than if it received no food at all.¹¹ The importance of salts has been dwelt upon by various workers, but no one has shown in simpler way, or in a more convincing form, the reasons for the value of salts in the economy than M. H. Fischer.¹² It seems to be true that the importance of various salts in the human organism is due to their effects upon the physical condition of the proteins of the body. They furnish practically no energy and yet are essential. Schaefer¹³ has said that the chemistry and physics of the living organism are essentially the chemistry and physics of the nitrogenous colloids, and Guyer¹⁴ wrote that what we call protoplasm “is really an aggregate of colloids holding water for the most part, in which are contained certain salts and non-electrolytes.”

These salts, it seems, are of intrinsic value because they change the affinity of the protoplasm for water, so that at one time when there is a relative lack of salts, the protoplasm loses water, and at another time they absorb water, and with it, food materials. In other words, we may suspect that the salts of the food are the agents which control to a large extent secretion and absorption. Certain salts, of course, are regulators of neutrality in the body, but that is but a part of the problem of secretion and absorption.

⁸Tachau: *Biochem. Ztsch.*, 1914 (65) 253.

⁹Starling: *Human Physiology*, 1912, p. 5.

¹⁰cf. Abt. *Jour. A. M. A.*, 1913 (61) 1275; also Grafe: *Deut. Arch. f. Klin. Med.*, (113) 1.

¹¹Starling: *Human Physiology*, 1912, 724.

¹²Fischer: *Oedema and Nephritis*, 1915.

¹³Schaefer: *Science*, 1912 (36) 289.

¹⁴Guyer: *Trans. Amer. Micros. Soc.*, 1911 (30) 145.

All of the various salts have perhaps different values in the economy. In Osborne and Mendel's experiments, the salts of sodium, calcium, and magnesium were used in the form of chlorides and phosphates, which indicate from the outcome of their work that these are the more important ones.

Certain salts such as many of the organic acid salts (citrates, for instance) act as alkalies in the body. They are diuretic and cathartic and this action is the result of withdrawal of water from the tissues. It is comprehensible that such salts tend to over-alkalinize the tissues. Salts of the inorganic acids on the other hand, tend to over-acidize the tissues when used in more than sufficient amounts.

The role of salts, while not sufficiently elucidated, has played some considerable part in medical literature. It has been held that goitre, pellagra, beriberi, scurvy, rickets, etc., are in part, or as a whole, dependent upon the content of the diet of certain salts. In no case has the proof been brought. Recently it has been suggested¹⁵ that (in the case of pellagra) the salts of silica are the active cause, but that the silica must be in a colloidal form.

Most interesting of all the suggestions, are those which have come of recent years and which concern themselves with the so-called vitamins.¹⁶ These substances cannot be classed as yet with either proteins, carbohydrates, fats or salts, though it seems possible that they may be salts in a colloidal state. Cooper¹⁷ is inclined to believe that these substances, formerly referred to as "antiscorbutic" are neither proteins, fats, or lipoids, and there is no evidence that they are carbohydrates. The term as first used by Funk was applied to certain substances of undetermined structure which occurred in the outer parts of grain. (It is this part which is removed during the polishing of rice.) It has been suggested that there are different vitamins which are active in preventing disease—one for beriberi, one for scurvy, one for pellagra, etc. They are present in food stuffs in such exceedingly small amounts that they cannot, it seems, have any caloric value and yet they seem to be essential. It has been suggested that they are in some way united with the part of the food in which flavor resides.

There seems to be but one general way in which to formulate the diet question, bearing in mind the fact that "a man who maintains his weight may be in excellent nutritive condition, but a child who does the same thing, is failing to grow."¹⁸ Each individual needs a different amount of food, which will vary according to his structure and surroundings; he will need protein, carbohydrate, fat and salts, in addition to water; he will need certain types of proteins, certain types of fats, and certain salts; and finally he may need other substances, which have perhaps to do with flavor, called generically vitamins, if he is to show physical growth.

¹⁵Cencelli: London Lancet, Apr. 17, 1915.

¹⁶Funk: Die Vitamine, Wiesbaden, 1914.

¹⁷Journ. Hyg., 1912 (12) 436.

¹⁸Osborne and Mendel: Carnegie Inst. Publication 156, 1911.

Variations in the Rate of the Heart Beat

QUESTIONS relating to the pulse rate (and the heart rate) are of ever-continuing interest, and everywhere clinicians discuss with eagerness the variations in the heart beat and attempt to draw conclusions which may be more or less valuable in proportion to the knowledge of the clinician of the physiology of the heart. It deserves saying that, even in the case of the most careful man, the knowledge will be limited because so little is known of the cause of the cardiac (and therefore the pulse) rate.

There are, however, a certain number of data that it is well to consider in discussing the pulse rate. First of these, is the fact that from the tachycardiac pulse of the infant there is a gradual decrease with age, from about 130 at birth to 85 at about the twelfth year, with a further decrease to an average of about 70 at twenty years. This average remains fairly constant up to say 50 years, after which time there is a slight increase. All this means is that there are *age variations* in the pulse. Chotak refers to these variations as growth period changes, and suggests that they correspond to a gradual increase of vagus tone. Perhaps they are due to changes in the motor functions of the heart. The difficulty with this idea is that we do not know what "vagus tone" is, nor what "changes of motor function" are.

Again there are individuals whose average pulse rates and heart rates are above, in some instances, or below, in others, the so-called normal average. There are persons who have a constant rate of 100. There are also persons whose rate is constantly below 70. Since the cause of the normal rate is not known, one can only surmise as to the cause of the variations. In certain cases there seems to be an anatomic basis for the tachycardias or bradycardias. Michailoff, for instance, found a neuroma in the dorsal cord of an individual who had shown a tachycardia since childhood and who lived to be 62 years old. In this case the cardiac rapidity may have been due, as Michailoff suggests to a mechanical pressure effect upon accelerator fibres, or it may have been due to some other factor.

Again there are differences in heart rate connected with sex, size, temperature (Loeb and Ewald), time of day and relation to meals (Weber), posture, etc. Geigel observed an average loss of 12 beats during recumbency and suggested that this was the result of increased cerebral pressure and vagus irritation.

In this connection the observations of Klewitz are exceedingly interesting. He observed an average loss of cardiac frequency of 19.9 per cent during sleep. He believes that this loss which may be greater under perfectly quiet conditions is the result of cutting down of afferent nervous impulses from the environment. He also says that a heart in which there is a compensated valvular lesion acts like a normal heart in respect to its recumbency losses, and that in decompensated the losses approach the normal in direct proportion as the lesion is compensation;—in other words, that the percentage decrease serves as an indicator of the degree of decompensation. In severe cases of cardiac decompensation the pulse rate during sleep may run above the waking normal. Such a heart cannot recover its tone during sleep as does a normal or compensated heart.

A considerable number of observations and experiments which are of in-

terest, have been made to elucidate the respiratory cardiac variations. The fact that there is an inspiratory quickening of the heart rate and an expiratory slowing is interesting and not understood. It has been suggested upon the basis of experimental work that these respiratory variations are the result of vagus impulses and that the respiratory types of arrhythmias which are observed in children, in nervous persons, and in convalescence from acute infectious diseases, are the result of a "certain lability of the vagus center." Such phenomena are, however, observed in perfectly healthy individuals (Putzig) who should possess, then, particularly "labile vagus centers." These so-called "vagus arrhythmias" (Pongs) form a cumbersome group. It includes the sinus arrhythmias of the respiratory type, the arrhythmia infantilis, those which appear during convalescence from infectious diseases. Tabora calls them the nervous arrhythmias. These respiratory variations were originally conceived as being due to purely mechanical effects produced by intrathoracic pressure changes. This was in the days when nervous influences were supposed to be supreme. Experimental work however has shown that this was not the case. Later it was considered possible that they were due to reflexes from the lungs to the vagus. Experimental work has not substantiated this conception nor that which considered the variations the result of reflexes from the respiratory muscles. Following these nervous explanations have come the chemical ones. One theory was that the respiratory frequency of the pulse was dependent upon the degree of oxygenization of the blood, because during ventilation of the lungs with oxygen the expiratory slowing was absent, and when the oxygen supply ran low the slowing reappeared.

Finally since it has been shown that the cardiac variations occur in complete absence of nervous influences, recent workers have fallen back on the theory that the variations are metabolic in origin, or that they are the results of temperature changes in the blood. Peterson and Gasser showed that the products from fatigued muscles increased the forces of systole with but slight slowing of the heart beat. Mansfeld showed that CO_2 does not increase the pulse rate. Other workers also have shown, apparently, that metabolites alone do not affect the rate of the heart and pulse.

Mansfeld's work indicates that temperature increases of the blood are essential. He showed that tetanization of the musculature of animals produces increases of temperature in the right heart, and with hot salt solutions injected into the femorals an evident "heat acceleration" of the heart was produced. The worst of it is that Mansfeld guesses that there are heat nerves in the heart which act in making with the accelerators a reflex arc.

It seems that in the insistence upon the necessity of nervous impulses the investigators are making a hard thing out of an easy one. It is certainly true that nervous impulses are useful to normal function, but that does not make them absolutely essential. It must be true that they influence metabolism by changing the rates of chemical changes. But when one can sever all nervous connections and still obtain all the usual effects (which usually appear more slowly) in their normal order it becomes evident that it is not necessary to theorize about nerve fibres and nervous impulses concerning which nothing is known. And when evidence is given that temperature variations may con-

dition heart rate, an essential factor has been found that needs no nerves to help it. In infants, metabolism is very rapid; the blood reaching the right heart is warmer than that which reaches it during quiet in the adult. The heart ought to be quicker. In the adult, exercise which increases metabolism reproduces the infantile conditions and the heart is quicker. Inspiration slows the influx of blood to the heart; expiration increases it, and so quickens the pulse, and the quickening is shown in the inspiratory phase because it has not the opportunity to produce itself sooner.

These remarks are not intended to eliminate the nerves. In the neurotic individual it is entirely probable that impulses reach the heart by way of the nerves and that depending upon which group is affected, the heart is slowed or quickened, by which we mean that the metabolism of the myocardium is increased or decreased. During such a process heat must be produced even though it is not appreciable. We know that very slight temperature changes modify deeply the changes in chemical solutions, and particularly in colloids. One would say then that the pulse rate variations are the results of metabolic changes in the myocardium which are brought about by temperature changes, and that these result from increased chemical activity in other parts of the body when heat enough is produced to cause the myocardial change, or in the heart itself, through the influence of the nerves, which increase (accelerators); or decrease (vagus) the rapidity of the chemical changes.

It is significant that Laurens reached the conclusion from his experiments on *Amblystoma* embryos that (in those animals) the rate of the heart beat is a logarithmic function of the temperature; that the acceleration of the heart beat is greater for lower temperatures than for higher; and that Van't Hoff's law for the velocity of chemical reactions in relation to temperature holds for the heart beat. For the present, these results of Laurens are of greater significance than those of Martin, Gruber and Lanman, which lead them to the conclusion that (in humans) the pulse rate was not dependent upon the temperature.

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—P. G. W.

The Function of the Spleen

IN speaking of the function of the spleen, one may recall the story of the German professor and the American student. The professor asked a member of his class to tell him what the function of the spleen was. The student, after some cogitation, said that he was sorry; he had known, but that he had forgotten. At which the professor threw up his hands and said: "Gott! only one man has known this, and he has forgotten!"

The correct conception of the role of the spleen is that it is in part a filter

for the blood, and in part a graveyard and reduction plant for the red blood cells. In it senile or dead red cells are filtered out, held and made use of by processes in splitting which free and modify the materials of which these cells are composed, sending part to the liver to be used as bile, and part to the bone marrow to be used in blood building. That this function is not an important one may be surmised from the results of splenectomy.

In a recent article, Asher and Ebnöther¹ report some experimental work which seems to have an important bearing upon the function of the spleen. They showed, first of all, that liver extracts have a decided hemolytic action, and that splenic extracts, though they may act hemolytically, do not act constantly or strongly in this way. But they also showed that by mixing liver and spleen extracts, hemolysis was far more active than it ever is in the absence of the splenic constituents. Whatever material is the active one is thermolabile and is not soluble in alcohol or ether. It is, they suggest, "either a ferment or a colloidal substance."

Their experiments with hemoglobin solutions show that in their action upon this substance, the organ extracts behave as they do toward red cells, that is, spleen extracts augment the lytic action of liver extracts, and that only spleen extracts will act in this way. Other organ extracts are inactive. Moreover, during the process of lysis (or rhesis) of hemoglobin, the process does not go to the extent of freeing the iron,—hemin is not obtained. The authors call attention to the fact that they have discovered another very interesting example of organic dependence or tissue correlation.

This work, if it be established, has a very useful practical clinical bearing. Heretofore splenectomy has been an experiment. It has been done as a last resort. But if in certain anæmic conditions it can be shown that abnormal hemolysis is fundamental, and that the anæmia is due to it rather than to a lack of production of blood cells or pigment, then it will be logical and not merely empirical to remove the organ which furnishes the normal augmentor of hemolysis. In such cases it may be that the liver itself is primarily over-active, or that the spleen is over-producing its augmenting substance.

Another indication of a very useful function of the spleen is to be found in an article of Launoy and Lévy-Bruhl.² These writers point out that in the case of infection with *spirochæta gallinarum*, splenectomy increases the grade of septicæmia but reduces the severity of the disease in that the toxic symptoms are much less marked than in other normal animals. Evidently the spleen is (in this disease at least) a very useful filter, but at the same time it is also a very active culture tube in which toxins are produced. It may perhaps be found that other infectious diseases depend for their severe symptoms upon this function of the spleen. The opposite point of view has been emphasized by Bardach, who found that splenectomized dogs were much less resistant to anthrax than normal ones. Bradford and Plimmer found that splenectomized animals inoculated with *Trypanosoma brucei*, died more quickly than others.

¹Asher and Ebnöther: *Zentralb. f. Physiol.*, 1915 (30) 61-64.

²*Ann. Inst. Pasteur.*, 1915 (29) 213.

Lesions of the Pancreas in Diabetes

THERE is, as Labbé, Laignel-Lavastine and Vitry¹ say, scarcely another question concerning which more controversy exists than that relating to the relationships of diabetes and anatomic changes in the pancreas.

Previous to the present era of discussion which began with Lancereaux in 1877, many writers had noted the presence of pancreatic lesions in cases of diabetes, and after this time, even to the present, lesions of the abdominal salivary gland are sought for in all cases in which death has occurred in the course of the clinical complex known as diabetes mellitus. The period of experimental work on pancreatic diabetes really commenced with Mering and Minkovski who produced the essential features of the disease by removing the pancreas. From this time the reports of histologic lesions in the pancreas began to be looked upon as an essential of the disease.

But at the same time, histologic studies of the pancreas in other clinical conditions than diabetes, in which there was no abnormal secretion of sugar, began to be made which gave evidence that the lesions commonly associated with diabetes were also present. Chronic interstitial pancreatitis involving the pancreas as a whole, only the tissue of the external secreting part of the gland, or only the islands, has been found associated with the most various conditions. In biliary lithiasis the most diverse lesions of the pancreas may exist, but sclerosis is the predominating one, existing in 80 of 100 cases (Mayo Robson). Weichselbaum has said that the use of alcohol predisposes to chronic pancreatitis. Chabrol has observed the same disposition in cardiac conditions. As a matter of fact, the most various factors have been reported as the causes of pancreatic changes. And nevertheless in very many instances, no matter what the type of lesion, glycosuria has been absent. In Schmidt's series of cases of diabetes, on the other hand, he found a normal pancreas in each of 8 cases, and in the majority of the other fifteen, there was so little change as to seem insufficient to account for the symptoms. Many writers, among them Hansemann, have only exceptionally found insular lesions in diabetes, though Opie and others considered insular lesions more or less essential. (It is of course to be remembered that the pancreas tissue may be abnormal in its response without showing any gross or microscopic lesion.) Hansemann considers the diffuse sclerosis which is analogous to that of the small red kidney, the essential diabetic pancreatic lesion.

Certain writers (Laguesse, Curtis, Gelle) believe that it is impossible to separate the insular lesions from the acinar ones, for, they say, histology seems to show that there is a continual transition from acini to islands and from islands to acini and that lesions involving the one should affect the other. As a matter of fact, the sections of pancreas made from post mortem tissues in the Cincinnati General Hospital, show as a rule diffuse changes rather than ones localized in one or another part of the glands.

Labbé, Laignel-Lavastine and Vitry have for several years made a histologic study of the pancreas tissue obtained from thirty-seven individuals who died of very diverse affections, and from nineteen who died of diabetes. In

¹Archives de med. exper., et d'anat. pathol., 1914 (26) 366.

both groups the same lesions were observed and so, it appears, the mere presence of certain types of histologic changes does not indicate the presence of diabetes; in other words, there is no pathognomonic pancreatic lesion in that disease.

—P. G. W.

Cardiac Murmurs

ONE sometimes observes the tendency in some practitioners to overemphasize the importance of cardiac murmurs. One more frequently observes the enthusiasm with which a student seizes upon a murmur at one of the cardiac valves as the one clinical fact upon which he proceeds to rest a diagnosis. And yet it may be that the murmur is the least important expression of physical deficiency. Cardiac murmurs have an essential value only when the whole cardio-vascular system is considered, and often only when the whole corporeal system of the individual is taken into account. Such remarks apply not only to murmurs but also to many other cardiac phenomena. It is therefore useful when a writer of experience calls attention to the fallacies of judging a patient by an isolated clinical phenomenon. Parsons-Smith,¹ for instance, says that murmurs must be investigated with care, in making interpretations from them, because a heart in which a murmur occurs may be a perfectly efficient one, and suited to the individual for whom it works. Also the amount of damage in a heart cannot be deduced from a study of the murmur alone. It is what a heart does, not what it sounds like, that indicates its value,—its strength or weakness. If at the end of a period of reasonable physical strain (or mental?) the heart has lost no tone,—shows no dilatation, it is a functionally good heart no matter how it whistles or blows. If it loses tone then it is not functionally fit, no matter if no abnormal sounds are heard. Changes in the size of a heart after exertion may be decided in many cases by use of the X-ray plate or, better, the fluoroscope.

¹The Practitioner, 1915 (94) 533.

—P. G. W.

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ORIGINAL ARTICLES

ON THE PRESENT STATUS OF THE ABDERHALDEN REACTION AND OF THE THEORY OF THE SO-CALLED ABWEHRFERMENTE*

BY J. BRONFENBRENNER, PH.D., PITTSBURGH, PA.

IN one of the papers of last year Oeller and Stephan¹ after having reviewed the work of different authors as well as on the basis of their own experience with the Abderhalden Reaction, came to the following conclusion:

"We think that further progress in the study of this subject is possible only on the condition that we throw overboard without hesitation all the clinical results obtained with the Dialysis Reaction up till now and begin the study anew.

"One must openly admit that, in spite of the fact that this method can give and has undoubtedly given under certain conditions biologically true results, the exact study of the subject is impossible because the errors in the results are fundamentally connected with the very technic of the method."

Whoever has had experience in performing the Abderhalden Test for diagnostic purposes in a routine laboratory will undoubtedly accept at least the second part of the above statement. Moreover a number of investigations originating from different laboratories continuously increase the evidence against the very nature of "Abwehrfermente" as understood by Abderhalden.

In spite of the fact that among those who were unable to confirm Abderhalden's findings we see the names of men very prominent in the field of research in immunity, Abderhalden usually attributed unsatisfactory results obtained by these investigators to the fact that they did not use all the necessary precautions prescribed by him for the carrying out of the test.

Abderhalden actually says that one should not attempt even to discuss the practical value of the test or its theoretical basis so long as one is unable to obtain satisfactory results in preliminary tests. He begins one of his articles² by saying:

"A method, whatever be its nature, can give comparable results in the hands

*From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.

¹Oeller and Stephan: Deut. Med. Woch., 1914, No. 31, p. 1557.

²Abderhalden: Munch. Med. Woch., 1914, No. 5, p. 233.

of different workers only after each individual investigator has mastered the test so as not to obtain contradictory results."

Abderhalden is quite right in what he says, but the main trouble with the Abderhalden Reaction is, that on account of its very nature it is impossible to obtain always results free from contradiction until the true nature of the phenomena involved is better known.

In the last two years I have conducted with my collaborators a number of investigations into the nature of Abwehrfermente of Abderhalden, as well as in the mechanisms of the Abderhalden Dialysis Method for diagnosis.

Before relating our results I would like to describe in short the principles involved in the test.

The experiments of Weinland³ have shown that the parenteral introduction of cane sugar is followed by the appearance in the blood of experimental animals of a specific ferment of the nature of invertin. Later, especially the experiments of Abderhalden and his numerous collaborators, have suggested that this apparent production of a specific enzyme following upon the parenteral introduction of foreign material is an expression of a general mechanism of protection on the part of the invaded organisms, and the ferments were accordingly called "Protective" (Abwehrfermente).

According to Abderhalden these ferments are strictly specific. They can be demonstrated in the blood of experimental animals within 24 hours⁴ after parenteral introduction of foreign substances and are quite independent from antibodies which may be produced simultaneously by this treatment of animals. In fact, the experiments of Weinland and of Abderhalden and his pupils seem to show that the production of specific ferments is an even more general process than the production of antibody. For whereas antibody can be demonstrated in the cases of parenteral introduction of substances of animal or plant protein origin only, provided this protein is foreign to the species, it is claimed that the specific ferments have been demonstrated not only upon the parenteral introduction of such substances, but also upon that of proteins of homologous and even autogenous nature, provided these substances are foreign to the blood ("blutfremd"). Moreover the parenteral introduction of substances like gelatin, pepton, cane sugar, or casein is proved by the Abderhalden school to produce specific ferments capable of attacking said substances both in vitro and in vivo. Thus the group of substances which can play the part of antigen in the production of antibody is included in that of the substances capable of causing the production of specific ferments, but is only part of it.

Another apparently fundamental difference between the nature of antibodies and specific ferments is the mode of their action. The antibodies, although directly responsible for the specificity of the protective processes in the body through their property of anchoring the antigen, do not in themselves present any active principle, and it is to the complement that Ehrlich and his school attribute the property of acting upon the antigen. The protective ferments of Abderhalden, however, are assumed to possess the property of directly digesting the antigen, and it is on the appearance of the products of such direct digestion in vitro that the diagnostic method of Abderhalden is based.

³Weinland: *Zeitschr. f. Biol.*, 1907, p. 279.

⁴Abderhalden: *Abwehrfermente*, 4th Ed., 1914, pp. 77-78.

The fundamental principle of the test is thus as follows: If the serum containing specific protective ferments is brought in contact in vitro with the substance which stimulated the production of given ferments in the body of the donor the ferments attack such substratum. The digestion, which results, is characterized by the appearance of soluble split products exhibiting the property to change the plane of the polarized light as well as the ability to dialyse through animal membranes.

Accordingly, Abderhalden suggested two procedures for the detection of digestion taking place in the test; (1) the so-called optical method, necessitating expensive apparatus and therefore, less widely used, and (2) the so-called dialysis method which is almost exclusively used in the diagnostic work and consists in the following procedure.

Serum of the patient in whom the presence of protective ferments is suspected as a result of possible invasion of his blood circulation by foreign substances (syncytial cells, cells of malignant growth, bacteria, etc., as the case may be) is placed in a specially selected dialysing thimble together with some of the material (substratum) which is suspected to have invaded the blood. Such thimble is then placed in a container with the water and the whole is incubated in a thermostat. If the serum of the patient contains the specific ferment in question, such ferment digests part of the substratum and the soluble products of digestion penetrate through the wall of the thimble into the outer fluid in the container. The presence of such protein split products in the dialysate is further ascertained by means of a chemical reagent "ninhydrin" specially adapted for this work. From the findings of the protein in the dialysate, one concludes that the digestion must have taken place during incubation.

Experience showed, however, that the test may be rendered valueless through even a slightest deviation from the rigid procedure. The most insignificant defect in thimbles or other parts of the apparatus or technic constitute a great source of errors in the results.

It is therefore essential that from the beginning to the end the technic prescribed by Abderhalden must be followed in all details. In our experiments we followed the procedure described by Abderhalden in numerous publications and added to it in a few instances some improvements in details provided these changes were directed towards the ends emphasized by Abderhalden himself. Thus, for instance, we included all the suggestions we could find in the direction of freeing the serum of hemoglobin, freeing placenta of blood, preventing bacterial decomposition of the contents of thimbles, rigid control of thimbles, uniformity of boiling with ninhydrin, etc.,—all of which improvements are thoroughly in accord with Abderhalden's conception of the method.

TECHNIC OF THE ABDERHALDEN TEST

Serum.—In collecting the blood serum one must guard against the following sources of error:

Since the test depends on the appearance of dialysable ninhydrin reacting substances it is most essential that such substances should not be present in the serum before its incubation with substratum. Fortunately in normal cases the amount of such dialysable substances in the blood is too small to complicate the test, but under certain pathological conditions in which protein katabolism

is involved (such as in cases of abscess formation, resorption of exudates or transudates, hemorrhages, etc.) the blood may contain considerable quantity of such substances. On the other hand even physiologically the amount of dialysable protein constituents of the blood is largely increased during and some time after the active digestion of food. The blood is therefore taken as long after the last meal as possible. And as for the pathological conditions in which the increased amount of dialysable protein constituents cannot be regulated at will, it is necessary to free the serum of such substances by a preliminary dialysis and only after having the serum so prepared, one can perform the Abderhalden Test, otherwise the test is not reliable.

It is very often emphasized by Abderhalden that the serum be absolutely free from hemoglobin.⁵ This is of especial importance in the optical method of diagnosis. As for diagnosis by the method of dialysis many authors have stated that the presence of hemoglobin in the serum often causes nonspecific reactions.⁶

If the serum contains red blood cells, even in a very small quantity, the outcome of the test is subject to doubt because such cells undergo hemolysis when the outer fluid penetrates inside the dialysing thimble and the hemoglobin as well as some of the other soluble protein constituents of the cells may cause the dialysate to react with ninhydrin in a nonspecific manner.

In short the procedure of collecting the serum for the test is as follows:

In cases of both pregnant and normal individuals 20-25 c.c. of blood is withdrawn aseptically preferably before breakfast from the median vein of the arm. A large Luer syringe and a large lumen needle are used for the purpose. The syringe and needle are sterilized by boiling, and allowed to dry thoroughly before use. Immediately after collection the blood is transferred from the syringe into sterile centrifuge tubes coated on the inside with paraffin and centrifuged at high speed; the serum is separated from the sediment by means of a sterile pipette and placed in a second sterile centrifuge tube for a repeated centrifuging. The clear serum is then separated and placed on ice until the time for using.

Selection of Thimbles.—It is known that colloids do not penetrate animal membrane. All natural proteins being colloids therefore are not dialysable. The slightest digestion of proteins, however, yields soluble substances, peptones, which are able to dialyse. If thus a natural protein, which is nondialysable is placed inside of an organic membrane surrounded by water and is acted upon by a proteolytic enzyme, the natural protein is changed in the process of digestion and its split products (peptones, amino acids, etc.) characterized by their ability to pass through the membrane, appear in the fluid surrounding the membrane. This can be ascertained by a biuret reaction applied to the dialysate. This selective relation of organic membrane to true proteins and their split products is made use of in the study of ferment action in general.

⁵In order to avoid breaking down of red blood cells, one must not place the whole blood on ice nor in the thermostat because red blood cells often undergo hemolysis at low temperature and if placed in the thermostat, the cells may undergo autolysis, either of which will render the serum unsuitable for the test.

⁶In our experiments we were most careful to avoid the presence of hemoglobin in the serum, in spite of the fact that our experiments as well as those of other workers (De Waele, *Munch. Med. Woch.*, 1914, p. 364; Lange, *Berl. Klin. Woch.*, 1914, p. 785, and others) could not detect any marked influence of hemoglobin (in the amount in which it may be present in the serum as a result of partial hemolysis) on the specificity of the test.

and Abderhalden puts it as the basis of his Dialysis Test for detection of "Protective Ferments."

It is evident, that the selective action of membranes is possible only on condition that the membrane is intact through all the surface of its contact with the natural protein. If, for instance, there should be even a slightest puncture in a membrane, the unaltered protein will go through. On the other hand, experience has shown that among the membranes used in dialysis, there exist wide individual variations in permeability to dialysable substances. Thus, for instance, of the two apparently identical membranes, one may let through peptones with great ease, whereas the other may let them through very little or sometimes even not at all. It is evident that in cases when the dialysis through the membrane is to be made the basis for drawing conclusions as to the physico-chemical nature of substances within it, it is imperative to eliminate this variability in the density of the wall of the membrane. With this in view Abderhalden has examined a number of different membranes and finally made his choice of dialysing thimbles made by Schleicher and Schüll and designated by the number 579-A. These thimbles show a comparative uniformity as to permeability by standard solutions of dialysing substances both of organic and inorganic nature. As the whole outcome of the Abderhalden Test, however, depends on this uniformity of thimbles as well as in their being free from mechanical injury, it is essential to always ascertain these qualities in individual thimbles before they can be used for the test proper.

Test for the Impermeability to Natural Protein.—The new thimbles are placed for a couple of hours in cold distilled water until they are completely penetrated by it and are comparatively soft and pliable.⁷ At this time each thimble is tested as to its efficiency on the one hand of holding back the natural protein and on the other of allowing the dialysis of soluble protein derivatives and split products with desired ease.

As a substance to test the impermeability of thimbles to true proteins, Abderhalden advocated the use of a 20 per cent emulsion of egg-white⁸ or of normal serum.⁹ Before using, each thimble is quickly sterilized by a short immersion in boiling distilled water (about 30 seconds). In placing the protein emulsion in the thimble one must be always very careful to deposit it as carefully as possible on the bottom of the thimble. This precaution is necessary because if even the slightest amount of protein is deposited on the outer walls of the thimble, the test will not be reliable, since the whole egg-white as well as

⁷It is essential to remember that at no time the thimbles are to be allowed to dry after they are first taken out for use. Dried thimbles are extremely fragile. In order to keep them moist, the thimbles are placed in a container with sterile distilled water which protects them also from mechanical injury due to handling, as the water softens all the possible shock to which the outer jar may be subject. Besides, if the thimble is once used, it may retain a trace of protein on its surface, and in case it is allowed to dry, the protein is subsequently washed out only with great difficulty. Moreover, during the time while the thimble is drying, bacteria which it contains on its surface, in multiplying, may close up some of the minute pores, from which it is very difficult to remove them subsequently.

All of these possibilities of injury to the thimbles are removed, however, if thimbles are properly washed after their use and kept under sterile water in presence of an antiseptic.

⁸In making the emulsion he advises to use very fresh eggs and in addition to carefully discard all the gelatinous part of the egg-white, using only the part which is very fluid and uniform. Infected egg-white will contain dialysable products of decomposition.

⁹It seems that the serum is more preferable in certain respects, because being more easily soluble than egg-white, it is easier removed from the surface of thimbles after their use by simple washing, whereas in case of the eggs, it is always necessary to use a soft brush and help the removal of protein by gentle rubbing during the washing. However, as the serum may sometimes contain dialysable substances, and thus may not represent sufficiently uniform natural protein for the purpose of testing the impermeability of the thimbles, we always are careful to dialyse such serum against running sterile distilled water in presence of thymol, and use only such serum, freed of dialysable substances, it might have contained for the test.

the whole serum even in very high dilutions gives positive biuret or ninhydrin tests. It is therefore advisable in case of doubt, to rinse the thimble on the outside with distilled water, so as to insure the absence of protein on the outer surface of the thimble. This is done by closing the open end of the thimble between the tongues of sterile forceps¹⁰ and pouring sterile distilled water from a wash bottle over it. The water must not be blown out from the wash bottle, for little by little the saliva collecting in the mouth-piece may penetrate into the bottle and contaminate the water both with bacteria and with protein.¹¹ After having placed egg-white or serum in the thimble, one places the latter into a glass receptacle containing sterile distilled water, covers both the contents of the thimble and the water in the outside receptacle with a layer of about 0.5 c.m. of toluol and transfers the whole in the thermostat for 16-18 hours.

In placing the thimble into the receptacle with water it is advisable to take care that the level of the fluid both inside and outside the thimble be the same, in order to prevent the evaporation of the water from the higher level through the wall of the thimble.

At the end of incubation the outer fluid is removed by means of a sterile pipette and examined by means of the biuret reaction for protein.¹² If the substance within the thimble is free from dialysable substances and if the thimble is of good quality, the result of such examination should be negative.

In short, the test of thimbles for impermeability to true protein is made as follows:

New thimbles are placed in sterile distilled water. After one hour's soaking each thimble is quickly immersed into boiling distilled water (for 30 seconds), cooled by rinsing with cold sterile distilled water and 5 c.c. of 20 per cent solution of egg-white¹³ is carefully placed by means of a sterile pipette on the bottom of the thimble. In order to prevent contamination of the edge of the thimble by protein, one may use the special funnel described by Malikjanz.¹⁴ Close the open end of the thimble by pressing it between the tongues of sterile forceps and rinse the outside with sterile distilled water. The loaded thimble is placed into a glass receptacle of Jena glass containing suitable amount of sterile distilled water to rise to the level of the fluid within the thimble. Both the contents of the thimble and outer fluid are covered with a layer of toluol about 0.5 c.m. in thickness and the receptacle is covered with a small round glass plate.¹⁵ After 16-18 hours 10 c.c. of the outer fluid is withdrawn by means of a sterile pipette¹⁶ which is introduced to the bottom of the receptacle through the layer of toluol.¹⁷ The dialysate is placed in a Jena test tube, 2.5

¹⁰In selecting the forceps one must be careful to use only those with plain tongues, not indented, so as not to injure the thimbles. For the same reason it is preferable to round up the ends of the tongues.

¹¹According to Abderhalden the saliva gives a positive ninhydrin reaction even in dilution of 1:1,000,000 with distilled water.

¹²Although in the test proper it is preferable to use ninhydrin for detection of protein in the dialysate, in case of testing the thimbles for impermeability to true proteins, it is preferable to use biuret tests. The reason for doing so is that the biuret is more sensitive to unchanged or very slightly changed protein, whereas the ninhydrin test is especially sensitive to the products of advanced digestion of protein, but less useful in early stages. (See Abderhalden, 4th Ed., p. 217.)

¹³One can use instead, normal serum, previously dialysed against running water. In this case one uses 2.5 c.c. of serum and dilutes it with salt solution up to 5 c.c.

¹⁴Malikjanz: Munch. Med. Woch., 1914, No. 23, p. 1287.

¹⁵This is done to keep out the dust as well as to prevent rapid evaporation of the toluol.

¹⁶It is absolutely necessary to use a chemically clean pipette for each test and avoid even the slightest traces of saliva to reach the fluid in the pipette.

¹⁷In order to prevent toluol from penetrating into pipette, it is advisable to close the free end of it tight with a finger while going through the toluol. The presence of a slight amount of toluol does not, however, seriously interfere with the subsequent test.

c.c. of sodium hydroxide solution of highest purity are added and the whole is thoroughly mixed by shaking from side to side.¹⁸ At this time one adds to the contents of each test tube 1.0 c.c. of a 0.2 per cent solution of copper sulphate, taking care that it should run along the wall of the test tube, which is held for this purpose in an inclined position. In this way the two solutions remain superimposed and in a short time there will be a more or less intense violet or pink coloration at the ring of contact of the two fluids if the dialysate contains protein and in such cases even the slightest trace of coloration indicates that the thimble must be discarded. In case however there is no coloration at the end of thirty minutes one may conclude that the thimble is impermeable to protein (unchanged) and may be used for the test provided it is permeable to derived proteins.¹⁹

Each thimble which is found impermeable to natural proteins is cleansed by means of a soft brush and left in running water. After one hour the thim-

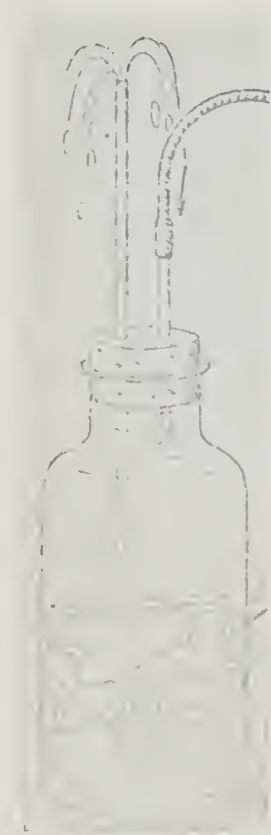


Fig. 1.—Apparatus for washing thimbles in running distilled water. Dialysing thimbles are seen at bottom of receptacle. (Abderhalden's *Abwehrfermente*, 4th Ed., p. 220.)

bles are transferred to distilled water and washing is continued until it is reasonable to believe that every trace of protein is removed.²⁰ Such thimble is then again immersed for about thirty seconds²¹ into boiling distilled water for the purpose of sterilization and the whole procedure is repeated exactly as in

¹⁸Sometimes at this point the solution becomes turbid, but this does not interfere with the test. It is essential to shake the fluid and not to mix it by closing the mouth of the tube with a thumb and tilting it over, for in doing so one introduces the substances from one's skin, which may give the biuret test.

¹⁹We found it very useful to use the precipitin reaction as recommended by Abderhalden for determination of permeability of thimbles to natural proteins, but of course for this test it is always necessary to have on hand a specially prepared precipitating serum, which is not always possible in diagnostic laboratories. Especially in cases when biuret gives doubtful results such control test is of value.

²⁰This is done in a specially constructed apparatus (see Fig. 1). If the water resulting from the washings of these thimbles should be examined at this time for protein, it should of course give negative reactions.

²¹One has to be careful not to boil the thimbles longer because boiling changes the physical properties of the texture of the thimble.

the case just described, except that instead of egg-white or serum one uses 2 c.c. of a 1 per cent solution of silk peptone (Hoechst) in distilled water, which is placed in the thimble with all the precautions mentioned and dialysed against distilled water. At the end of dialysis of 16 to 18 hours in the incubator one removes carefully 10 c.c. of the dialysate as before, only at this time in addition to the biuret test also the ninhydrin test is performed. For this purpose one introduces in a clean dry test tube 0.2 c.c. of a 1 per cent solution of ninhydrin by means of a carefully calibrated 1 c.c. pipette,²² and adds to it 10 c.c. of the dialysate withdrawn from the container with the precautions just described, and the whole is brought to boiling. It is essential that the boiling be regular and the length of boiling uniform in all the comparable cases. To insure these

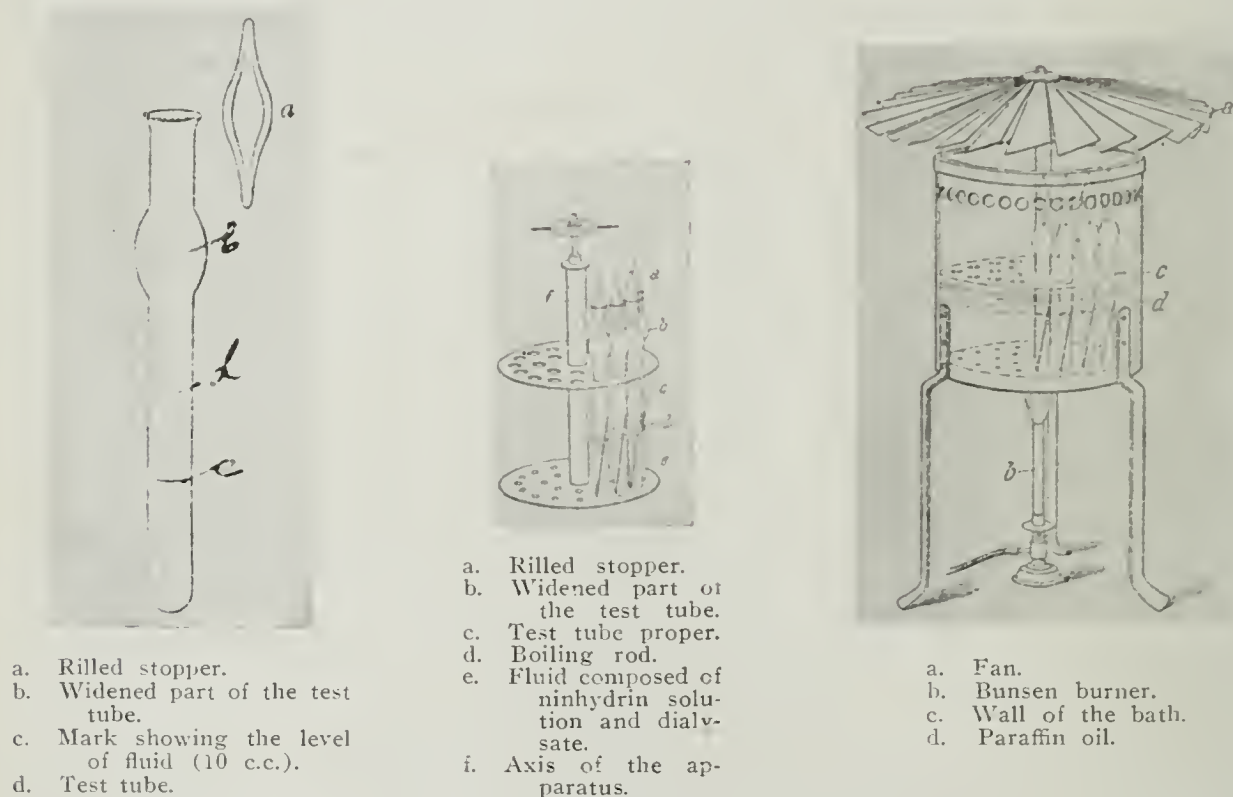


Fig. 2.—Paraffin oil bath (Abderhalden's *Abwehrfermente*, 4th Ed., p. 233).

two requirements one places in the tube a clean dry boiling rod²³ and continues the uniform boiling exactly for one minute from the time when the air bubbles first appear in the wall of the test tube.²⁴

Care should be taken not to allow the fluid in the test tube to run over during the boiling. As soon as one minute of boiling is over, the tube is removed from the flame and the boiling rod is withdrawn.²⁵ Reading of the results

²²One must be careful to deposit all of the necessary amount of ninhydrin solution on the very bottom of the test tube and not on its walls, so as to assure the presence of absolutely equal amounts of ninhydrin in all comparable reactions.

²³Boiling rods are very carefully washed, boiled in distilled water, dried at 60 to 70° C. and kept aseptically in a closed glass tube until needed for use. It is essential not to handle these rods with fingers, but always with sterile forceps. In drying, one must guard against overstepping the limit of 70° C., for at higher temperature the wood may carbonize and the brown color diffusing into fluid interferes with the reading of the color reaction.

²⁴The uniform boiling can be accomplished on an open Bunsen flame if at the moment that boiling begins, the tube is placed half way outside the flame, so as to allow only a small surface of the bottom of the tube to remain in contact with the periphery of the Bunsen flame. This allows to continue without interruption and without the fluid boiling over. Wherever it is possible it is best to use for boiling the paraffin bath described by Oeller and Stephan (*Deut. Med. Woch.*, 1913, p. 2505) and improved by Abderhalden (*Abwehrfermente*, 4th Ed., p. 232), which allows to boil the dialysate of all the thimbles in one test at exactly comparable conditions.

²⁵Boiling rods should be discarded each time, and new rods treated as described in the footnote 23, should be used with each test.

shall not be attempted until the fluid is cooled, which at room temperature takes about thirty minutes. At this time one may notice that the intensity of coloration may vary with different dialysates. This is due to variations in permeability of the thimbles. The thimbles, which do not let through but very little peptone (which is indicated by a low intensity of blue coloration obtained by boiling of respective dialysate with ninhydrin) should be discarded as unsuitable for the test. Only such thimbles as allow *liberal* dialysis of protein derivatives should be carefully washed, sterilized by 30 seconds boiling and stored for future use in sterile distilled water. The container in which the thimbles are stored must be sterilized before the thimbles are placed in it. In order to prevent subsequent infection of the thimbles it is advisable to place a few drops of chloroform in the bottom of the receptacle and a liberal amount of toluol on the top of sterile distilled water so as to bring the lower surface of the glass stopper in contact with toluol. When needed for use the thimbles should be removed by means of sterile forceps and with all the precautions necessary to avoid bacterial contamination of the contents of the receptacle with the thimbles. After using, each thimble is washed with all the precautions described above, boiled again for thirty seconds and replaced in a receptacle with other sterile thimbles by means of sterile forceps.²⁶

Substratum.—Inasmuch as the protective ferments are supposed to be specific, the ideal substratum would be represented by a pure protein. In practice, however, this is possible only in exceptional cases mainly in experimental work, as in diagnostic work, we are dealing with organs which are a conglomeration not only of different proteins within the cell, but of most different cells within the organ. In order to approach the ideal, however, it is evident, that the more the given organ is freed of nonspecific elements, the better. We will mainly consider the preparation of placenta substratum, as except for minor points the same procedure is true for other organs.

First of all the tissue which is to be used as substratum, is to be freed from all the blood and lymph. Lange and Strooman²⁷ for instance, report cases where the patients having hematomata reacted with the substratum containing blood, but did not react with similar substrata free from blood, thus the presence of blood complicating the question of specific digestion of tissue, according to these authors.

It is advisable for the same reason to remove as much of the connective tissue, blood and lymph vessels, and in general, all of the nonspecific tissues as possible, preserving, however, all the specific elements of the organ. In cases where it is very important to establish an absolute specificity of the reaction, and where the absolute uniformity of substratum is impossible to be obtained, it is advisable to make a control using the contaminating tissues alone instead of the specific substratum in a parallel test.

In order that the tissue shall present all its typical characteristics, it is very important to use only fresh tissues before any autolysis or bacterial decomposition has set in. It is for this reason also advisable to discard the tissues of the cases in which a protracted agony preceded death. Also the tissues which came in contact with disinfectants should be discarded for similar reasons. In

²⁶It is advisable from time to time to change the distilled water in which the thimbles are stored and always refill with toluol so as to allow the stopper to come in contact with it.

²⁷Lange and Strooman: Deut. Med. Woch., 1914, No. 13, p. 635.

general, all the precautions possible must be taken so as to prevent any biologic changes in the tissues previous to their being used for preparation of substratum. As in the case of serum, also in the preparation of substratum one must be sure that the final product is free from dialysable ninhydrin reacting substances. To assure this, the substratum in the process of preparation is thoroughly extracted with boiling water until the water is absolutely free from ninhydrin reacting substances. Only such substratum can be used for the test. As the preparation of substratum from fresh tissue takes from three to five hours, and as it is impossible always to obtain fresh tissues, and as on the other hand, it is impossible to preserve fresh tissues any length of time without changes, it is necessary to find means to keep a supply of prepared substratum on hand. It is therefore very important to handle the substratum once prepared with the utmost precaution in order not to introduce bacterial infection. If this precaution is not taken the presence of bacteria in large amounts in itself introduces a complication of the presence of nonspecific protein, and in addition, the infected substratum may develop soluble ninhydrin-reacting substances.

Keeping the above considerations in view, one proceeds in the preparation of the substratum as follows:

The tissue is collected as soon as possible after delivery (in case of placenta), after operation (in case of various tumors), or as soon as possible after death (the organs from the people killed in accidents are preferable to those dying from disease). In case of placenta the whole organ is carefully transferred to the laboratory, free blood is pressed from it and the intact organ is washed through the umbilical vein by turning running water into the blood vessel until the tissue is practically colorless. Then the placenta is carefully dissected and all the vessels and connective tissue are removed. The resulting placental tissue is carefully cut up in small pieces by means of a meat grinder and placed in a deep precipitating jar into which a large amount of sterile distilled water is added.²⁸ After a thorough stirring the tissue is allowed to settle out. The supernatant fluid is then syphoned off, the water is thoroughly pressed out from the tissue, and a new quantity of sterile distilled water is added, the contents of the jar are stirred up and the tissue allowed to settle. This is repeated several times until the examination of tissue under the microscope does not reveal any blood cells. At this time the tissue is practically white in color.²⁹

In case of lung tissue, the procedure is absolutely identical. As for the other tissues it is preferable to cut them in very small pieces previous to washing, and still better, to freeze the piece of tissue and cut it up by means of a microtome into very thin pieces. Treated in this manner tissue of any structure can be freed from blood.

When the tissue is absolutely free from blood it is immediately coagulated by boiling.

In order to do so a large amount of distilled water (*not tap water*) (about

²⁸Lange (Biochem. Zeitsch., 1914, lxi, p. 193) advises the use of physiological salt solution for washing because the water leaves erythrocytic stromata in the tissue. This was, however, not found necessary as no stromata could be found in the placenta tissue after good washing, in spite of careful examination by many authors (Domarus and Barsieck, Munch. Med. Woch., 1914, p. 1553).

²⁹Although the requirement is that the tissue be white which is very important, Abderhalden warns, however, against the use of decolorizing substances, like H_2O_2 for instance, for the white color of the tissue is important, not by itself, but only as an indicator of the absence of the blood, which can be accomplished only by thorough washing.

one hundred times the amount of tissue) is brought to ebullition in a large enameled casserole (*not a glass beaker*) and the tissue is quickly immersed in the boiling water. One may add to the boiling water 1-2 drop of glacial acetic acid per liter of water to accelerate the coagulation.³⁰ The boiling must continue until the complete coagulation of protein (about thirty minutes). After this the water is decanted³¹ and the tissue is transferred into another casserole³² with the same amount of boiling distilled water. The boiling is continued for ten minutes without further addition of acetic acid. Distilled water is thus changed 5 or 6 times, which usually suffices to free the tissue of all soluble substances. In order to see whether this is accomplished one transfers the tissue in a comparatively small amount of boiling distilled water (3 to 5 volumes) and after a short boiling, 5 c.c. of this water is collected through a hardened filter into a test tube, 1 c.c. of 1 per cent solution of ninhydrin is added, and the mixture is boiled for one minute.

If the tissue is free from soluble protein derivatives, the contents of the test tube remain colorless.³³ If there is the slightest violet coloration upon cooling, it indicates that the substratum still contains some of the soluble ninhydrin reacting material, and therefore, the boiling in excess of distilled water has to be repeated until every trace of such substances is removed. At this point the coagulated tissue is carefully examined against a white background and all the particles of tissue which are not absolutely white are discarded.

Now the tissue is ready for use in the test. It is boiled once more in order to kill bacteria which might have been introduced by the manipulation and the sterile tissue is placed by means of sterile forceps in a sterile glass jar with a ground glass stopper. The layer of the tissue in the jar is covered with sterile distilled water and on the top of it one places sufficient toluol so as to allow the glass stopper to touch it and thus seal the contents of the jar.

Above it was ascertained that the substratum does not contain ninhydrin reacting substances. However important this requirement is, it is not less important to ascertain that during this treatment, the tissue retains its property to undergo digestion by an appropriate specific ferment. It is necessary to ascertain in other words that if the substratum in question be placenta for instance, that this placenta substratum can be digested by the serum of a pregnant individual.³⁴ With this in view the tissue is placed in a dialysing thimble with normal and pregnant serum respectively and a regular test with all necessary controls is performed as is described further.

In most cases, if the above technic is carefully followed, and if placenta (or any other tissue) is carefully prepared, this test will show that the substratum retains its property to react with specific serum by giving off dialysable

³⁰Some writers are not sufficiently clear upon this point. According to some of them one has to "add to the tissue 100 volumes of distilled water and boil for ten minutes." In doing so, one is really cooking and extracting the tissue (even though a small amount of acid is present) instead of quickly coagulating all the coagulable protein. It is essential to add the tissue to the water only after the latter has been brought to ebullition.

³¹If the amount of tissue is small, or in case one is dealing with bacterial substrata, it is advisable to centrifuge the boiled substrata before decanting the water, in order to avoid possible loss of material.

³²It is important not to do it the other way in order to avoid the burning of the tissue on the bottom of the casserole.

³³If the tissue still contains soluble ninhydrin reacting material the contents of the tube would turn violet.

One must always cool the tube after boiling under running water, because the ninhydrin reaction, negative immediately after boiling, may appear upon cooling.

³⁴Another control is to be made to show that the same placenta is not digested by the serum of normal or of individuals suffering from different pathological conditions.

ninhydrin reacting substances, whereas it will not react with a normal serum. A still better test of the specific qualities of the substratum consists in the following. A suitable quantity of the substratum is placed in a thimble with a specific serum and a regular test is performed. If the substratum is good, there will appear in the outer fluid in due time, ninhydrin reacting substances. Now one removes the substratum from the thimble, washes it very thoroughly from the serum with cold water and subsequently subjects it to boiling until the boiling water again gives no color reaction with ninhydrin, and finally uses the same substratum again for the test with a normal instead of specific serum. If in this instance the reaction is negative, the substratum is satisfactory.

As for the quantity of substratum to be used for the test, it is evident that all depends upon the degree of care exerted in the preparation of substratum. If all of it consists of specific elements, a much smaller amount will suffice for the reaction, than in the case, when most of the specific cells are washed away, in which case a far greater volume of substratum will be needed to supply the necessary amount of specific elements.

The suitable amount has to be therefore determined experimentally by using a given amount of normal as well as specific serum in parallel series and combining them with varying amounts of substratum. The largest amounts of substratum will probably give positive reaction with normal as well as specific serum, whereas the smallest amount may give negative results with both sera. Between the two extremes will be a zone of quantities which are reasonably safe to use in the test.

It is evident that the estimation of the specific value of substratum is very important and each new stock of substratum should be properly standardized.

After the value of the substratum is established, it is ready for use in the routine test.

From now on the substratum must be handled with the most rigid aseptic precautions. When needed for the test, part of the tissue is removed with sterile forceps and once having been taken out the tissue is never placed back into the jar. As the tissue is being taken out, the jar must be filled with toluol to take its place.³⁵ If kept and handled aseptically, the substratum keeps indefinitely.

GENERAL TECHNIC

Test Proper.—Before the routine Abderhalden test is undertaken, one must ascertain that all the apparatus, as well as suitable biological material and chemicals are at hand. It is very important not to set out to do the test before everything is prepared, so as not to have to leave the work between the manipulations.³⁶ As on the one hand the outcome of the ninhydrin test is very easily influenced even by the slightest presence of acid fumes, and as on the other hand the thorough asepsis is essential in all the stages of the test, Abderhalden recommends to reserve for such work a special light room, free from dust or fumes. It is essential that neither chemical nor especially, bacteriological work should at any time be performed in the same room. For the same reason it is

³⁵This precaution is necessary to prevent any possible decay of small particles of tissue, which may adhere to the walls of the vessel.

³⁶One of the essential requests of Abderhalden is that before performing the test, the worker not only learns thoroughly the technic, but actually "lives through" the test, in other words a thorough knowledge of fundamental phenomena involved is absolutely necessary. (See *Abwehrfermente*, 4th Ed., p. 261.)

advisable to set aside an incubator for this work separate from the bacteriological incubator.

For the reason that the ninhydrin test is subject to the influence of the reaction, it is essential to use all through the test glass which is as free as possible from alkalis.³⁷ From the discussion of the nature of the test it is evident that impurities are of no less importance. It is therefore most essential that all glass used in the test be chemically clean, as well as dry and sterile.

The water used in the work must be distilled freshly and sterilized.³⁸ Finally it is important not to undertake too much work at one time, because the test requires the utmost care and cannot be reliably performed in an automatic manner. Abderhalden therefore warns against performing more than 5 or 6 tests in one day. Some of his pupils, however, claim that only one test can be performed with all the necessary care and attention.³⁹

Immediately preceding the test proper, it is necessary to ascertain that the substratum have not changed, namely that it is still free from soluble ninhydrin reacting substances.

A portion of substratum necessary for the performance of the whole test with its controls, is removed from the container by means of sterile forceps, placed in a thoroughly clean and dry test tube, and boiled with 4 or 5 volumes of sterile distilled water.⁴⁰ After about 5 minutes of boiling during which care is to be taken not to burn the tissue, one places about 5 c.c. of the water (in which the tissue was boiled) through a small piece of hardened filter paper into another thoroughly clean test tube and by adding 1 c.c. of 1% ninhydrin solution and subsequent boiling (as described before) one ascertains whether the tissue is satisfactory. Even slightest trace of violet color indicates that the tissue must be re-extracted.⁴¹

If the tissue is found to be free from soluble ninhydrin reacting substances, it is washed in sterile distilled water so as to remove any chloroform or toluol which it may contain and dried between the folds of sterile filter paper. Such tissue free from excess of water is ready to be used in the test.

One places a desired number of tested dialysing thimbles in a corresponding number of thoroughly clean dry sterile Erlenmeyer flasks and introduces by means of sterile forceps about 0.5 gr. of the tested and dried substratum inside of each thimble. It is essential that the tissue should all fall to the bottom of the thimble, so as to be able subsequently to cover it with serum. At this time one holds up the thimble with sterile forceps (not the same with which the tissue was distributed) and introduces 1.5 c.c. of patient's serum so as to cover the substratum.⁴² Now the thimble is withdrawn from the flask, closed on its open end by means of long forceps⁴³ and washed with sterile distilled water to insure the absence of protein on the outside of the thimble.

After having thus been loaded, and washed, the thimbles are placed into a

³⁷Abderhalden therefore advises the use of Jena glass exclusively.

³⁸Commercial distilled water or spring water is unsuitable for use even after sterilization on account of bacteria protein it may contain.

³⁹Schawlow: Munch. Med. Woch., 1914, No. 25, p. 1386.

⁴⁰If the substratum happens to contain large pieces, it is essential to break them up before the boiling.

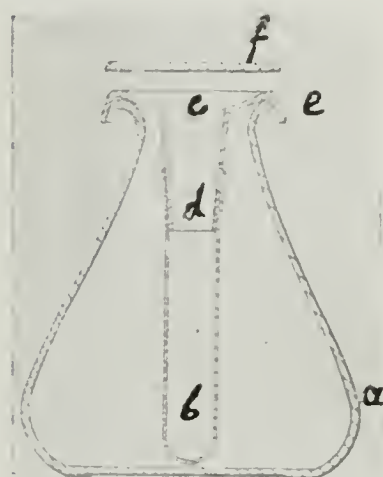
⁴¹See discussion under *Preparation of Substratum* for further instructions.

⁴²Care must be taken not to allow even a trace of the serum to be deposited on the edge of the outer wall of the thimble.

⁴³It is a mistake to close the thimble between two fingers as recommended in some American texts and Abderhalden warns specifically against such procedure repeatedly.

new set of Erlenmeyer flasks containing each 20 c.c. of sterile distilled water,⁴⁴ the content of the thimble, as well as the water outside is covered with a layer of toluol which would cover the greater part of the thimble (about 2/3 of its height) and the flasks are placed in the incubator having been covered with clean dry glass discs.⁴⁵

After 16-18 hours of incubation⁴⁶ the flasks with thimbles are removed from the incubator. Now each thimble is carefully withdrawn from the surrounding fluid and placed in a fresh (clean, sterile and dry) Erlenmeyer flask and the dialysates are examined for protein by means of the ninhydrin test. Namely 0.2 c.c. of 1% ninhydrin solution is placed in each of several clean, dry test tubes of Jena glass, to each of them 10 c.c. of respective dialysates is added⁴⁷ and the mixture is boiled for one minute with all the precautions described before.⁴⁸



- a. Erlenmeyer flask.
- b. Dialysing thimble.
- c. Upper opening of the funnel.
- d. Lower opening of the funnel.
- e. The brim of the funnel.
- f. Glass disc (cover).



View of the glass funnel.

Fig. 3.—Longitudinal section through the flask with funnel in place.

In order to be able to compare the results on the basis of the intensity of coloration obtained with ninhydrin, it is essential not to allow any variation in boiling, for inequality in the concentration of fluids during the boiling may lead to serious errors in the interpretation of the results.⁴⁹ It is therefore advisable

⁴⁴It is best to sterilize the Erlenmeyer flasks with the water in them immediately before the test. Care should be taken to let the water cool to the room temperature before using the flasks for the test. In sterilizing, the flasks should be plugged with cotton, which is not to be removed until it is actually used in the test.

⁴⁵In connection with these manipulations Abderhalden calls attention to at least two possible sources of error.

First, in subjecting the thimble to washing after it has been filled, one can by mistake introduce some of the water in the thimble, which is extremely undesirable, because it disturbs the uniformity of concentration, which is very important in dialysis.

Secondly, since both the flask and the thimble are almost full, it may happen that in careless handling the fluids outside and inside the thimble may become mixed, which destroys the very principle of the test. It is therefore that Melikjanz suggested (*Munch. Med. Woch.*, 1914, p. 1287) the use of a glass funnel which fits tight into the thimble as well as over the edge of the Erlenmeyer flask and thus allows the filling of the thimble with substratum and serum directly in the flask in which dialysis is to take place, and at the same time obviates the possibility of mixing the fluids. (Fig. 3.)

⁴⁶It is up to the man who does the test to decide upon the duration of incubation, 16 or 18 hours is about the best time, as it allows to make use of night hours. It is essential, however, in having decided on a certain duration of incubation to duplicate it exactly in all experiments.

⁴⁷Here again each fluid is to be withdrawn by means of a separate sterile pipette plugged with cotton and in general all the precautions described above in testing the permeability of thimbles are to be most carefully followed.

⁴⁸It may seem superfluous to call attention to the necessity of numbering the flasks, thimbles and test tubes with corresponding numbers so as to be able to identify the results, as the necessity of doing so is obvious.

⁴⁹This will be further discussed under *reading of the results*.

to boil all the tubes at the same time and with the same intensity, which can be accomplished by the use of an oil bath referred to before.⁵⁰

Having performed the ninhydrin test in each of the dialysates of the series, one is through with the test. Now, after having read the results, one washes thoroughly each thimble in running *cold* water, then in distilled water and after a short boiling (30 seconds) stores the clean, sterile thimbles away in the container with sterile distilled water, as described previously, under toluol.

DISPOSITIONS OF THE DIAGNOSTIC TEST

The peculiarities of the test discussed in previous paragraphs, its extreme sensitiveness to different conditions interfering with the proper manipulations and extreme importance of preserving the biologic ingredients of the test in good condition, necessitates a certain number of controls, which must accompany each test, or each series.

First of all, as it was mentioned before, even previous to beginning the test proper, one must ascertain the fact that the substratum is free from soluble protein derivatives which by themselves could react with ninhydrin. In spite of the fact that only such substratum, which answers the requirement of being free from soluble ninhydrin uniting substances is used for the test, it is still recommended to control such substratum again as follows:

Parallel with the test proper, one tested thimble is filled with 0.5 gr. of substratum⁵¹ and 1.5 c.c. of physiological salt solution, placed in an Erlenmeyer flask containing 20 c.c. of sterile distilled water, the contents of thimble, as well as the fluid outside are covered with liberal amount of toluol and in general the whole procedure prescribed above for the test proper is followed up carefully.

Second control consists in ascertaining whether the serum is suitable for the test. As was stated above, the serum may contain varying amounts of dialysable ninhydrin reacting substances. Since the presence of these substances cannot be detected otherwise than by actual dialysis, it is necessarily parallel with the actual test to dialyse the serum by itself and if such control will show that serum contained dialysable substances, the results of the actual test will have to be interpreted accordingly (see below).

In setting up this control one places 1.5 c.c. of the serum of the patient⁵² on the bottom of a tested thimble, introduces such loaded thimble into an Erlenmeyer flask containing 20 c.c. of sterile distilled water, covers the contents of thimble as well as the outside fluid with toluol and generally repeats all the manipulations (including the ninhydrin test on dialysate) prescribed above for the test proper.⁵³

⁵⁰In using the oil bath, the boiling has to be continued 3 minutes instead of only one minute as in case of boiling on a free flame.

⁵¹The quantity of substratum may vary slightly according to the outcome of preliminary standardization.

⁵²For each serum used in the test (the unknown as well as the known control sera) one must carry this control.

⁵³Although separately each of these two control tests (on substratum and on serum) are usually sufficient to determine whether the ingredients contain dialysable ninhydrin reacting substances, some authors (quoted by Domarus and Barsieck, *Munch. Med. Woch.*, 1914, p. 1553) claim that negative ninhydrin test on dialysate in these controls does not always exclude completely the presence of dialysable substances. They claim that owing to extreme dependence of ninhydrin test on concentration, the outcome of each test separately may be negative, whereas the combined dialysable substances of both—serum and substratum—may give a nonspecific (summation) positive ninhydrin

The third control consists in ascertaining that the substratum has retained its specific properties, namely that while not reacting with normal serum, its combination with specific serum will result in appearance of dialysable ninhydrin reacting substances. It is evident that such control consists in two tests—one in which the substratum is allowed to undergo digestion with known normal and in the other with known specific serum.

In performing these control tests one places equal amounts of substratum in each of two tested thimbles and introduces 1.5 c.c. of known normal and specific sera respectively in each. From now on one proceeds the same as in other tests—namely, places the thimble in Erlenmeyer flask with distilled water, covers the contents of the thimble as well as the outer fluid with toluol and incubates until subsequent examination of the dialysate 16-18 hours later. If the substratum retained its specific properties and of itself does not contain soluble protein, the dialysate of the test containing known normal serum will give a negative ninhydrin test and the one with the specific serum will give positive ninhydrin test.⁵⁴

In short each series of diagnostic tests is to be composed of following individual units:

TABLE I.

Thimbles.	Character of the serum used.		Amount of serum.	Amount of substratum.	Amount of NaCl Solution.	Ninhydrin Results as Expected.
1	Unknown ⁵⁵	(Test)	1.5 c.c.	0.5 gr.	?
2		(Control)	1.5 c.c.	Negative
3	Known Specific	(Test)	1.5 c.c.	0.5 gr.	Positive
4		(Control)	1.5 c.c.	Negative
5	Known Normal	(Test)	1.5 c.c.	0.5 gr.	Negative
6		(Control)	1.5 c.c.	Negative
7	No Serum ⁵⁶		0.5 gr.	1.5 c.c.	Negative

reaction. They offer therefore a very useful modification in controlling the test—namely to place as before, serum and substratum in separate thimbles, but now introduce both thimbles into one flask and eventually test the dialysate.

Another way to exclude the possibility of summation reaction is suggested by Kjergaard (*Zeitschr. f. Immunitätsforsch.*, Orig. xxii, No. 1, p. 31). This author recommends in each case to make a duplicate test with inactivated serum and thus demonstrate that the dialysable protein in each case appears as a result of specific activity of proteolytic enzyme, and not from the combination of dialysable substances of substratum and serum.

⁵⁴As stated before, each of the known sera has to be separately controlled in a thimble to show that they are free from dialysable substances reacting with ninhydrin.

⁵⁵The test with unknown serum is repeated with each of unknown sera and thus the thimbles 1 and 2 may be multiplied as many times as demanded by the number of unknown cases. It is, however, advisable not to undertake too many examinations at one time.

In order to avoid the error in results due to possible imperfection in technic which may pass unnoticed, it is advisable to make in duplicates the test proper (thimble 1 on the chart above) with each of unknown sera.

The controls (thimbles 3-7 incl.) are set up only once with each day's series.

⁵⁶In cases when one is working with substratum which cannot be obtained free from contaminating protein, one must naturally have an additional control for the contaminating substratum. For instance, in case of substratum being bacteria, the whole series must be repeated with sterile culture medium instead of bacterial substratum; or in case of substratum being tuberculous lung tissue the whole test with all the controls must be repeated with the normal lung tissue in place of tuberculous.

READING OF RESULTS

General Considerations.—The occurrence of specific digestion in the thimble is judged by the appearance of dialysable protein derivatives in the fluid surrounding the thimble. This is ascertained as stated before by a ninhydrin test.

The procedure of the ninhydrin test is purely empirical. It is possible that in the future a better knowledge of its intimate mechanism will result in the further improvement in its application. In the meantime a few facts known about it seem to be very essential to keep in mind if one desires to place reliance on the results obtained.

First of all, as it was said before, the acidity or alkalinity of the media interfere with the test in giving atypical color reaction which may obscure the reading.⁵⁷ Such atypical reactions may in reality be due to impurity alone, but at times they may mask a true test which may be present at the same time. It is therefore imperative to repeat the test in all cases where the color reaction is atypical.

Another extremely important element in this empirical test is the concentration. In this respect it is essential to control two conditions. First, the relative concentration of ninhydrin in the media in which the protein is looked for, and for this reason the solution of ninhydrin has to be made very carefully;⁵⁸ it has to be, moreover, very carefully measured and placed on the bottom of the test tube, as mentioned before, so that none of it will be lost, and add to it exactly 10 c.c. of the dialysate in each test.

Secondly, it seems that the concentration of ninhydrin reacting substances in the dialysate may cause a great deal of variation in the outcome of the test, and it is therefore essential to cover the contents of Erlenmeyer flasks (both within and without the thimbles) with toluol, so as to prevent any possible evaporation and unequal condensation of the fluids in different tests.

The two conditions mentioned above control the true relative concentration of ninhydrin and the substances with which it gives the color reaction.

Considering, however, the intensity of the final color reaction one must remember that there is still another factor which can influence it to a great extent—the condensation in boiling. The mixtures containing the same amount of ninhydrin as well as protein will show the reaction of different intensity if one is boiled longer than the other. According to Abderhalden⁵⁹ it is suffi-

⁵⁷The color reaction due to the acidity or alkalinity of the media is usually reddish-brownish-yellow. One can judge better about the specificity of this color reaction where it occurs in concentrated solutions, but when atypical color reaction is very weak it is often impossible to differentiate it from a weak protein test with ninhydrin. However, the difference can be made more evident if one produces a normal ninhydrin test with protein and dilutes it so as to match the intensity of the color reaction which is in doubt. The comparison of the two color reactions in equal concentrations of color will disclose a true violet tinge in a typical ninhydrin color reaction, which is absent in an atypical one.

⁵⁸In preparing the solution of ninhydrin from the dry commercial preparation one proceeds as follows: The contents of a tube (0.1 gr.) are transferred as carefully as possible without loss or bacterial contamination into a measuring flask. Since the ninhydrin cannot be transferred quantitatively, it is necessary to wash out the remaining substance by means of warm sterile distilled water. Ninhydrin does not go in solution very readily, unless in warm water; it is therefore advisable to add some sterile distilled water and place the flask in an incubator for a few minutes. When all of the ninhydrin is in solution one cools it down, fills the flask with distilled water up to the mark (10 c.c.) and places such solution on ice, till further use. Ninhydrin can be kept in solution fairly well especially as only 10 c.c. of it is made at one time. In general, however, bacterial infection may destroy it as well as the excessive exposure to light—it is therefore necessary to keep it in dark flask and in dark cool icebox and handle it aseptically.

⁵⁹Abderhalden: *Abwehrfermente*, 4th Ed., p. 223.

cient that one of the test tubes be removed from the flame during boiling just for a moment, and it will show different intensity in color reaction from others. It is therefore advisable to use the oil bath, referred to above, and thus ascertain exactly comparable conditions of boiling for all the dialysates in the test.

Considering all the factors which may contribute to obscuring the results of ninhydrin test, one must not forget that ninhydrin is very sensitive to even smallest quantities of protein and their derivatives (peptones, polypeptides and amino acids) so that even the slightest amount of saliva, perspiration, etc., which might be introduced in dialysate during numerous manipulations preceding the boiling with ninhydrin, may cause a typical, though nonspecific ninhydrin reaction.⁶⁰

Finally in looking at the results of the ninhydrin test, one must never use artificial light. Each test tube must be taken out of the rack and examined individually against a white background both through the thickness as well as through the depth of the fluid in the test tube.⁶¹

INTERPRETATION OF RESULTS OBTAINED

In testing the unknown serum (see Table I, Thimbles 1 and 2) the ninhydrin test on respective dialysate may give one of the four possible combinations.

TABLE II.

	Character of the Dialysate.	Color Reaction.	
Case I.	The dialysate from the serum alone (control)	Colorless	(neg.)
	The dialysate from serum and substratum (test)	Colorless	(neg.)
Case II.	The dialysate from serum alone (control)	Colorless	(neg.)
	The dialysate from serum and substratum (test)	Blue	(posit.)
Case. III.	The dialysate from serum alone (control)	Blue	(posit.)
	The dialysate from serum and substratum (test)	Blue	(posit.)
Case IV.	The dialysate from serum alone (control)	Blue	(posit.)
	The dialysate from serum and substratum (test)	Colorless	(neg.)

Case I. (Table II.)—Both the test tube containing the dialysate from the thimble with serum alone, as well as that with serum and substratum give negative reaction with ninhydrin. If all the precautions of the test have been followed⁶² this result indicates that there is no protein split products in the di-

⁶⁰It is therefore essential not to handle the thimbles or substratum otherwise than with sterile forceps; all glassware is to be absolutely clean, free from organic as well as inorganic impurities, dry and sterile; the pipettes used in the test must be plugged with cotton, absolutely clean, dry and sterile as well as the rest of the glassware used.

⁶¹It is evident that in order to obtain comparable results, it is advisable to select test tubes of equal diameter as well as thickness of the glass wall. In cases where the differences in the intensity of reaction are very small, this consideration attains great importance.

⁶²There are two points that are of especial moment considering the above outcome of the test. One, that the thimble is not too tight, and does let through the peptones if they are present. Although each thimble must have been tested before and its fair permeability must have been established, it is nevertheless advisable in each case of negative results in the test proper to subject the thimble in question to an additional test. In doing so, one washes the thimble very thoroughly and repeats another time the test for its permeability to silk peptone with all the precautions prescribed for such a test above.

The other condition to be definitely established before the result is taken to be of diagnostic value, that is, the requirement that the substratum retain its specific properties, that is to say, when placed in a thimble with serum which is known to contain the ferments in question this same substratum will be digested.

This query should be answered by the positive control (designated 3 and 4 on Table I), but one can also make an additional control test by removing the substratum from the thimble, washing it very carefully, boiling it and setting it to digest with a known specific serum. One should obtain a positive ninhydrin test on the resulting dialysate if the substratum is satisfactory.

alysates tested. In case of the dialysate from the serum alone, as we have stated it before, such outcome is the desirable one. As for the test proper, the absence of protein split products in dialysate signifies that the digestion did not take place in the thimble and this in turn means according to Abderhalden's conception of the test that the serum did not contain the ferments necessary to digest the substratum.

Case II. (Table II.)—The test proper gives a positive ninhydrin reaction and the control on the serum alone—negative. If all the controls accompanying the test (see Table I, Thimbles 5, 6, and 7) give expected results, the above outcome of the examination of unknown serum means that it contained specific ferment digesting the given substratum.⁶³

Case III. (Table II.)—A third possible outcome of the test may be that the dialysate from the thimble containing the serum alone (control), as well as dialysate from the test proper, in which the thimble contained both the serum and the substratum, both give a positive ninhydrin test.

In this case the appearance of dialysable substances in the thimble containing the serum and substratum is only conditional, because, as the control shows, also dialysate from the serum alone (without the addition of substratum) gives a positive ninhydrin test. Before any attempt is made to interpret this result it is advisable to establish definitely the cause of the presence of ninhydrin reacting substances in this control dialysate, for according to the cause the interpretation may vary.

Assuming that the technic was perfect, and that no protein could have been introduced in dialysate by the faulty manipulation (as for instance soiling the outside of the thimble with serum, while introducing it in the thimble, or contaminating the distilled water in the flask with saliva, while placing it by means of a pipette, or depositing organic matter on the outer wall of the thimble by handling it with fingers instead of forceps, etc.), there are two other possible reasons for the presence of protein in this dialysate: either the thimble was permeable to unchanged serum, or the serum contained dialysable products before the test.

Although the thimble was supposed to be tested and found to be impermeable to natural protein, it is still possible that in further manipulation in placing into or withdrawing the thimble from the receptacle in which thimbles are kept, such thimble might have been punctured in handling with forceps. In order to establish this point it suffices to make a coagulation test on the dialysate, and if it contains unchanged serum, the thimble is to be discarded and the whole test repeated with a good thimble. If, however, the dialysate does not contain coagulable protein, it means that the thimble was not punctured and that the substances giving the ninhydrin test are of the nature of dialysable derivatives and must have been present in the serum before the test.

Having established that the ninhydrin test on control dialysate is due to

⁶³In order to be absolutely sure of the specificity of this digestion, Abderhalden advises to work always with more than one substratum. For instance, if the serum to be tested is suspected to contain specific ferments against carcinomatous tissue, he advises to examine it also against placenta for instance or if pregnancy is suspected, it is well to examine the serum also with lung or carcinomatous tissue. In case of positive findings with the specific substratum (as this Case II illustrates) such additional control if correctly carried out, gives additional strength to the result obtained in the main test.

the presence of dialysable protein split products in the serum, one must compare the intensity of this reaction with that obtained on dialysate from the thimble containing both the serum and the substratum.⁶⁴ If the latter is decidedly more intense, the outcome of the reaction is still considered satisfactory and a positive diagnosis is rendered.⁶⁵ If, however, the intensity of reaction in both cases is nearly equal, the diagnosis cannot be rendered and the test must be repeated.

In cases, however, where it is impossible to obtain the specimen of blood free from dialysable ninhydrin reacting substances (as for instance if the patient is running a high temperature, or in general in cases where there is marked active catabolism of protein) it is necessary to recur to a special method suggested by Schlimpert and Issel. By this method⁶⁶ the serum is dialysed against running distilled water, until free from dialysable ninhydrin reacting substances, and only then it is examined according to usual procedure of Abderhalden.

Case IV. (Table II.)—It is possible finally, that the test of unknown serum will show a positive ninhydrin reaction on a control dialysate (serum alone) and a negative reaction on dialysate of the test proper (serum and substratum). This result is to be interpreted as follows:

First of all it is necessary to ascertain that the thimble in which the test proper was performed is not too tight, for it is possible that the failure to obtain the ninhydrin test in the dialysate from the test proper (serum and substratum) could be due to impermeability of the thimble to dialysable protein.⁶⁷ This is done as described before, by thoroughly washing the thimble and testing it subsequently with silk peptone. If the thimble is found impermeable, the whole test is to be repeated. If the thimble is found permeable, it is evident that digestion did not occur during the incubation, because the serum did not contain specific ferments.

As to the reason for the positive ninhydrin test obtained with the control dialysate, one must proceed as in the Case III just described; namely, first to ascertain whether the presence of ninhydrin reacting substances in dialysate was due to imperfection of the thimble. If coagulation test on dialysate is positive, one concludes that the thimble is punctured and discards it. If the coagulation test is negative, the thimble is not punctured, and the ninhydrin reaction must have been due to the presence in the serum of dialysable ninhydrin reacting substances.⁶⁸

SOURCES OF ERRORS IN PERFORMING THE TEST

Personal experience has shown us that if the necessary precautions are carried out and if all the controls required by the nature of the test are made, there

⁶⁴Abderhalden: *Abwehrfermente*, 4th Ed., p. 273.

⁶⁵In our own work we prefer not to attribute diagnostic value to the differences in intensity of color in ninhydrin test, because even slightest changes in concentration (brought about by imperfect boiling or by loss of even small portion of ninhydrin on the wall of the test tube) during the test may produce the difference in the intensity of color reaction sufficiently great to cause erroneous diagnosis.

⁶⁶Schlimpert and Issel: *Munch. Med. Woch.*, 1913, p. 1758.

⁶⁷Although the thimble must have been tested before and must have been found permeable for silk peptone, it is possible that the subsequent boiling in order to sterilize it, might have rendered the thimble impermeable.

⁶⁸Such cases, where the serum alone gives off into dialysate ninhydrin reacting substances, which disappear upon the addition of tissue, have been described by several authors and are attributed to absorption of such dialysable substances by substratum.

is no reason why one should not obtain good results with the Abderhalden test. It is evident from the preceding paragraphs, however, that the amount of detail is so voluminous and the number of elements, outside of the test proper, interfering with the proper outcome of examination so great, that it is not unlikely that many unsuccessful attempts to use the Abderhalden reaction can be accounted for in the manner in which Abderhalden does it, namely that the men performing the test did not master the methods sufficiently, before rendering their judgment of its value.

Many authors have stated that the test is too difficult to be adopted for general use. There is nothing in the technic of the test to justify such a statement. True, as was said just before, the amount of detail preliminary and accompanying the test proper is very great, possibly much greater than in other serological methods, but there is nothing in the nature of manipulations that would place a competent serologist in difficulty. Provided, one has time and material necessary to comply with the requirements of the test—only those who are novices in serology may make mistakes.

However, since the field of application of the Abderhalden reaction for the time being seems to be so broad, a great number of men with most varied preliminary experience and with very scant bacteriological and serological experience have rushed into collecting statistical data, instead of learning first the principles not only underlying the test proper, but the many sided manipulations as well.

One cannot but agree with Abderhalden when he says that those performing the test must not only know the procedure, but must as he puts it "live through it," or, as we understand it, those who never before did careful serological work, must first become serologists and bacteriologists, so that a great number of errors which may be due to their inability to work aseptically *by habit* (and not only because it is so prescribed in a given paragraph about Abderhalden test) may be eliminated. As important as these *habits of a trained serologist* may be in other instances, they are especially important in the Abderhalden reaction, where the technic of the test is so crude and ingredients so impure that the whole attention of the investigator must be disengaged from mere manipulations in order to be concentrated on careful and as extensive as possible control of the test proper.

I perfectly agree with Ebeler and Löhnberg⁶⁹ who say that as the "Abderhalden reaction is getting better known and as its technic is being improved by joint efforts of different investigators, it becomes so complex that its usefulness in diagnostic laboratory is growing more doubtful."

However, even those who have a suitable training which guards them from making mistakes in technic find that certain amount of mistaken diagnoses is unavoidable. The reasons for such unavoidable mistakes originate from the very nature of the method. Thus, for instance, in spite of all the care taken in selecting and testing of thimbles, one can never be sure of the fact that the thimbles were perfect during the test, and strictly speaking, one should not consider the test finished or the results obtained final until the thimble in which the test was made is retested again and found to be still perfect. For it often happens

⁶⁹Ebeler and Löhnberg: Berl. Klin. Woch., 1915, p. 319.

that a thimble, perfectly satisfactory at the preliminary test, is found useless subsequently. Thus for instance, boiling of the thimble may change the physical properties of its texture, so as to render it impermeable to silk peptone. Such change in permeability of the thimble cannot be discovered before the test, it is only through the critical analysis of the test already performed, that one can come to suspect the change in the permeability of a given dialysing shell. It seems that such changes do not take place at once (especially if care is taken not to boil the thimbles too much) but, according to a number of reports, even the most careful boiling if repeated a number of times may change certain thimbles. It is for this reason that some authors⁷⁰ recommend as routine precaution to discard the thimbles after they have been in use for about a month.

Another possible change in thimbles is that of becoming permeable to unchanged serum through mechanical injury, even so slight that it may often pass otherwise unnoticed. In order to avoid as much as possible the errors due to such changes in thimbles Abderhalden suggests that the stock of thimbles on hand can be repeatedly tested at least every two weeks. Such procedure certainly does not eliminate the possibility of changes in permeability, yet it adds a great deal to the already considerable amount of labor connected with the carrying out of the test.

Another group of errors which cannot be eliminated in spite of very careful procedure is that connected with the use of ninhydrin as indicator in the test. Not only such factors as faintest alkalinity of the glassware,⁷¹ or the length and intensity of boiling the ninhydrin solution with the dialysate⁷² (which can be regulated as described in the preceding pages), but also factors like dust and fumes for instance cannot easily be eliminated or can come to interfere with the test already begun.

Still another source of difficulty not easily controllable, is the serum, when it contains dialysable protein derivatives. As mentioned before, it is preferable in all such cases to dialyse the serum against sterile running water. This procedure is however sometimes very unsatisfactory—first for the reason that serum may contain bacteria which under the conditions of experiment multiply very rapidly; secondly, the dialysis against distilled water greatly weakens the activity of complement.⁷³ In order to obviate this difficulty we suggest to titrate the complement in each dialysed serum before using it for the test proper, and to activate it if necessary by the addition of guinea-pig complement.

MODIFICATIONS OF THE "METHOD NOT INVOLVING THE THEORY OF ABWEHRFERMENTE"

Recognizing these shortcomings of the technic a number of investigators attempted to obviate the errors in results by substituting better methods in such parts of the technic which were not amenable to proper preliminary control.

⁷⁰Schawlon: *Munch. Med. Woch.*, 1914, p. 1386.

⁷¹Setjen and Fraenkel: *Munch. Med. Woch.*, 1914, No. 9; also Abderhalden: *Abwehrfermente*, 4th Ed., p. 289.

⁷²Abderhalden: *Abwehrfermente*, 4th Ed., p. 225.

⁷³The important role of complement in the Abderhalden test is beyond any doubt and even Abderhalden himself acknowledges the fact without, however, being ready to interpret the nature of the influence exhibited by it. (See *Abwehrfermente*, 4th Ed., p. 154.)

Thus Michaelis and Rona⁷⁴ have suggested to eliminate the use of thimbles. Namely, the authors place the serum together with a substratum in a test tube, and after the necessary incubation at 37°, they centrifuge the contents of the tube, separate the liquid, precipitate it by means of ferric hydroxide, filter off the precipitate and make a ninhydrin test on the filtrate.

The extreme ease with which the proper outcome of ninhydrin test is influenced by most varied uncontrollable factors⁷⁵ has been instrumental in directing a great deal of efforts of different investigators towards finding a more reliable method for detection of protein in dialysate. Already Abderhalden himself⁷⁶ recommends to control the ninhydrin test by the Van Slyke method of determination of amino nitrogen in dialysate. Griesbach,⁷⁷ Abderhalden and Fodor⁷⁸ and many others employed microkjeldahl for the same purpose. On the other hand a number of authors tried to find another indicator to be used directly in place of ninhydrin, but so far only Matzkievitch⁷⁹ reports results which seem to be satisfactory. This author is using colloidal gold as the indicator and claims that by this method he obtains a distinct reaction with 1 c.c. of peptone solution of 1:30,000.

In addition to these modifications, a few methods were also worked out in our laboratory. Our methods also permit to dispose with the use of both ninhydrin and thimbles and in addition also permit to use the serum containing dialysable ninhydrin reacting substances without preliminary dialysis of the serum. These methods are, however, based on a conception of the reaction vitally different from that proposed by Abderhalden and will be discussed later.

CRITICAL ANALYSIS OF THE THEORY OF "ABWEHRFERMENTE"

The modifications of the technic of the Abderhalden test based on elimination from the test of thimbles and ninhydrin will undoubtedly simplify the method.

The experiments conducted in this laboratory, however, showed quite conclusively that the most serious and unavoidable mistakes in the Abderhalden test are due not to the use of thimbles or ninhydrin, but to the difficulty in controlling the exact quantitative relation between the substratum and the serum. On the one hand we have found that different samples of a given substratum differ amongst themselves as to their specific value. As we have stated before, such differences are in part due to the method of preparation and the amount of specific elements retained in substratum as compared to the amount of non-specific contaminating material in it. We found, however, that the variations in specific value of similar substrata are far greater than the limits of variations in technic of their preparation. In other words, two placentas, for instance, selected and prepared with exactly the same care may show different titers when standardized against a known specific serum. Whereas one placenta will react with 1.5 c.c. of a given specific serum in any amount above 0.1 gr., another sample of placenta substratum may be suitable only above the doses of 0.3 gr.

⁷⁴For description of the method, see Michaelis, *Deut. Med. Woch.*, 1914, p. 429. This method was recently improved by Van Slyke and Vinograd, *Proc. Exp. Biol. and Med.*, 1915, p. 126.

⁷⁵Bisgaard and Korsbjerg: *Deut. Med. Woch.*, 1914, p. 1367.

⁷⁶Abderhalden: *Abwehrfermente*, 4th Ed., p. 311.

⁷⁷Griesbach: *Munch. Med. Woch.*, 1914, p. 979.

⁷⁸Abderhalden and Fodor: *Munch. Med. Woch.*, No. 14.

⁷⁹Matzkievitch: *Deut. Med. Woch.*, 1914, p. 1221.

On the other hand we found that even if the same substratum is used, the titer of it differs with different sera. Thus, for instance, a given substratum may react with 1.5 c.c. of a pregnant serum *A* already in the amount of 0.15 gr., whereas in case of pregnant serum *B* it is necessary to have fully 0.4 gr. of the same placenta to obtain a definite reaction. At the same time we noticed that if the amount of substratum is sufficiently increased, any normal serum will react. Here again, different sera may react with different amounts of the same substratum (for instance, a male serum *A* will give off dialysable ninhydrin reacting substances if combined only with more than 1 gr. of a given substratum, whereas a male serum *B* will give off ninhydrin reacting substances if combined already with 0.4 gr. of the same substratum).

Quite apart from the difficulty of finding suitable quantitative relationship between the amount of serum and substratum to be used in the test, *the fact that it is at all possible to obtain a positive Abderhalden reaction with a male serum while observing all the precautions required by the test by merely slightly increasing the amount of placenta*, raises another serious question. If according to Abderhalden the appearance of ninhydrin reacting substances in the dialysate is due to the digestion of substratum in vitro by specific ferments present in the immune serum only, *how then is it possible for normal serum to digest the same substratum without the intercurrent of specific ferments?* It is evident that every serum must contain the ferment in order to be able to digest the substratum. Moreover, since normal serum may thus apparently digest in one instance excess of placenta, in another excess of carcinomatous tissue; in the third, tuberculous lung, etc.—it is necessary to admit either the existence in all the sera of all sorts of specific ferments, or the opposite—that there are no specific ferments present in the immune sera. The latter is the conclusion suggested by a number of authors. Flatow,^{80 81 82} Herzfeld,⁸³ Kjaergaard,⁸⁴ Plaut,⁸⁵ and others have thus been able to obtain apparent digestion of substratum by the ferments of nonspecific nature, present in any normal serum.

These investigations throw a new light upon the complexity of the nature of the Abderhalden reaction, or at least they show conclusively that, *while the reaction may be relatively specific within certain quantitative limits, it ceases to be outside of these limits*, depending entirely on mechanical adsorption. Whether or not these experiments can sufficiently explain all the discrepancies in the results of various investigators, they show that certain quantitative manipulations may bring about in a nonspecific way, phenomena closely resembling the Abderhalden reaction, thus questioning the basis of the Abderhalden theory, according to which the cleavage products appear solely as a result of the *specific digestion* by ferments. On the other hand the specificity of the ferments responsible for the digestion of substratum can be questioned on the basis of the experiments of De Waele,⁸⁶ who succeeded in demonstrating the presence of the ferments by means of the Abderhalden reaction already a few minutes af-

⁸⁰Flatow: Munch. Med. Woch., 1914, p. 468.

⁸¹Flatow: Ibid, p. 608.

⁸²Flatow: Ibid, p. 1168.

⁸³Herzfeld: Bioch. Zeitschr, 1914, p. 103.

⁸⁴Kjaergaard: Zeitschr fur Immunitatsforsch., Orig., 1914, xxii, p. 31.

⁸⁵Plaut: Munch. Med. Woch., 1914, p. 238.

⁸⁶De Waele: Zeitschr. fur Immunitatsforsch., Orig., 1914, xxii, p. 170.

ter the parental introduction of the foreign protein into experimental animals, an interval hardly sufficient for the production of new *specific* ferments.

These results corroborate the previous findings of Heilner and Petri⁸⁷ who concluded from the rapidity with which the ferments appeared as a result of the parenteral introduction of the protein that this must be a case not of new formation of such ferments, but of specific activation "Arteinstellung," of pre-existing ferment. De Waele, indeed, suggests the identity of this ferment with antithrombin.

Apart from the explanation of the Abderhalden reaction on the basis of mechanical adsorption or of activation of pre-existing ferments, a number of investigators attempted to study the phenomena underlying the Abderhalden test from the standpoint of immunity. Since it was established by several workers that the complement played an important part in the reaction, the simplest explanation of the Abderhalden test would be the identification of the substances responsible for the specificity of the Abderhalden test with the antibodies. No definite proof, however, of such an identification has been offered in the numerous publications on the subject. At the same time, Abderhalden states definitely that upon the parenteral introduction of foreign protein, independently of any antibody that may be produced simultaneously, specific protective ferments are formed. He comes to the conclusion of co-existence of two processes mainly on the basis of the fact, that, as his experiments have suggested, during the immunization of the animal its serum seems to acquire a new property to cleave the substratum directly, whereas the antibody, although directly responsible for the specificity of protective processes in the body through its property to anchor the antigen, is known not to attack it directly. It is to the complement that Ehrlich and his school attribute the property to act upon antigen.

However, the experiments of Stephan,⁸⁸ Hauptmann,⁸⁹ Bettencourt and Menezes⁹⁰ as well as our own experiments,⁹¹ suggest that the two processes might after all be closely related, as the ferments of Abderhalden seem to lose their activity upon heating at 58°, and once thus inactivated can be restored to action by the addition of any fresh serum (complement?).

The part played by complement in the activity of the ferments was finally recognized by Abderhalden, but he hopes to be able to find an explanation for this without identifying his protective ferments with the antibodies of Ehrlich.⁹²

This question of relation between protective ferments of Abderhalden and immune bodies of Ehrlich was extensively studied in my laboratory. Our experiments speak against the existence of specific ferments,⁹³ since even sera of highly immunized animals failed to digest directly the protein used for their immunization, although they gave at the same time a positive Abderhalden test.⁹⁴ We have shown that the dialysable substances appearing during the test do not originate from the substratum. It was found, on the contrary, that the fer-

⁸⁷Heilner and Petri: Munch. Med. Woch., 1913, p. 1530.

⁸⁸Stephan: Munch. Med. Woch., 1914, p. 801.

⁸⁹Hauptmann: Munch. Med. Woch., 1914, p. 1167.

⁹⁰Bettencourt and Menezes: Compt. rend. Soc. de biol., 1914, p. 162.

⁹¹Bronfenbrenner: Jour. Exper. Med., 1915, xxi, p. 221.

⁹²Referring to it he says: (see Abwehrfermente, 4th Ed., p. 154). "Nothing would be more unjustified than, on account of this parallelism, quickly to invest all the results obtained with names from the realms of immunity. For analogous phenomena need not be identical; moreover, complement, amboceptor, etc., are terms of which, in rare cases only, we have as yet a clear conception."

⁹³Bronfenbrenner: Proc. Soc. Expo. Biol. and Med., 1914, xii, p. 3.

⁹⁴Bronfenbrenner: Jour. Exper. Med., 1915, xxi, p. 221.

ments responsible for the cleavage of protein during the reaction in pregnancy are not specific; that they are present in every fresh male serum as well as female; that the protein attacked by these ferments is not that of the substratum but that of the serum of the patient, the Abderhalden reaction thus recording the result of the autodigestion of the patient's own serum.⁹⁵

As to the mechanism of this autodigestion of the serum, we evolved from our experiments the following explanation. Proteolytic ferments are present in every fresh serum, but are normally inhibited by some antitryptic constituents of the blood.⁹⁶

Removal of serum-antitrypsin sets free the normal trypsin of the blood, which in turn digests some part of the serum itself. Such removal of the antitrypsin may be accomplished *in vitro* by two apparently independent processes. One nonspecific,—a simple adsorption and filtering out of the inhibiting substances,—which would explain the results of Plaut,⁹⁷ Peiper,⁹⁸ Heilner and Petri,⁹⁹ who succeeded in obtaining a positive Abderhalden test by mixing the serum with substances like kaolin, starch, barium sulphate, etc., and on the other hand it would explain the results of Kjergaard, Flatow and our own, where the ninhydrin reacting substances appeared when normal serum was mixed with excess of substratum. The other, an apparently specific process, in which the falling out or inactivation of the inhibiting substances appears to be the result of the change of the colloidal conditions of the media, resulting from the specific combination of the antigen of the substratum with the antibody of the patient's serum,—a reaction which is identical with that recorded also by the stalagmometer in the experiments of Ascoli. The serum deprived of antitrypsin undergoes autodigestion, which is evidenced in the Abderhalden test by the appearance of dialysable substances.

This interaction between the substratum and the specific serum is in general comparable to the interaction between antigen and antibody in other immunity reactions, inasmuch as, apart from appearance of dialysable substances, the substratum in the Abderhalden test seems to undergo changes identical with those of erythrocytes when acted upon by hemolytic amboceptor; namely, the substratum anchors the specific constituents of the immune serum, thus becoming sensitized.¹⁰⁰ Such a sensitized substratum was found to be able to induce autodigestion in any fresh normal serum (complement), and thus the important findings of Stephan¹⁰¹ as to the part played by complement in the Abderhalden test can be easily explained. The heated serum containing only antibody (as the normal proteolytic ferments were destroyed by heat) retains the property of sensitizing the substratum. Upon the addition of guinea-pig complement a sensitized substratum, by removing the serum antitrypsin from the guinea-pig complement, liberates the proteolytic ferment in the guinea-pig serum, which in turn may digest the globulin not only of the serum of the guinea-pig, but also

⁹⁵Bronfenbrenner: *Proc. Soc. Exp. Biol. and Med.*, 1914, p. 7. These findings were confirmed by Eggstein (*Journ. Am. Med. Assn.*, 1915, p. 735).

⁹⁶According to Schwartz the lipoids of the blood are responsible for its antitryptic properties (Schwartz: *Wien. Klin. Woch.*, 1909, p. 861). Jobling and Peterson (see *Jour. Exper. Med.*, 1914, ix, p. 239) recently confirmed the antitryptic action of lipoids.

⁹⁷Plaut: *Munch. Med. Woch.*, 1914, p. 238.

⁹⁸Peiper: *Deut. Med. Woch.*, 1914, p. 1467.

⁹⁹Heilner and Petri: *Munch. Med. Woch.*, 1913, p. 1530.

¹⁰⁰Bronfenbrenner, Mitchell and Schlesinger: *Bioch. Bull.*, 1914, p. 386.

¹⁰¹Stephan: *Munch. Med. Woch.*, 1914, p. 801.

that of inactivated human serum, thus giving rise to dialysable substances. It is thus possible that the complement fixation phenomenon is merely another expression of the same reaction which by Abderhalden is recorded through the ninhydrin test. Considered from this point of view, the Abderhalden theory does not seem to contribute anything essentially new to the explanation of the protective processes which take place in the body as a result of parenteral introduction of protein, but simply offers a new indicator, by which the existence of the protective substances can be recorded in vitro.

DIAGNOSTIC METHODS BASED ON NEW CONCEPTION OF THE NATURE OF ENZYME ACTION IN THE BLOOD

Although we do not assert to have found definite proof that the nature of the "Defensive Ferments" is identical with that of the antibody or amboceptor, the results of our experiments seem, nevertheless, to contribute additional evidence to the effect that a considerable amount of parallelism between the two undoubtedly exists.

Thus, remembering the important role played by complement in the Abderhalden reaction we tried for instance to find whether the known property of antibody to sensitize the antigen at a low temperature, which excludes the activity of complement, is also true for the Abderhalden reaction. We found that there was not only no dialysis in the test containing placenta and the pregnant serum when the temperature was low, but also that the substratum as well as the serum underwent changes absolutely similar to those we should have expected if we had used, instead, a hemolytic amboceptor and corresponding erythrocytes, namely, the serum lost its specific elements, and the substratum became changed in such a way that is acquired property to give up dialysable ninhydrin reacting substances when mixed with any fresh serum (complement?); in other words, the substratum became sensitized.

Tables below (III and IV) show it quite conclusively:

TABLE III.
The Abderhalden Reaction Is Arrested at 0° C.

Pregnant human serum.		Normal male serum.		Control.
3 c.c. serum, 1 gm. placenta.	1.5 c.c. serum, 0.5 gm. placenta.	3 c.c. serum, 1 gm. placenta.	1.5 c.c. serum, 0.5 gm. placenta.	5 c.c. 1% silk peptone.
In glass tube at 0°C. for 16 hrs.	In thimble at 0°C. for 16 hrs.	In glass tube at 0°C. for 16 hrs.	In thimble at 0°C. for 16 hrs.	In thimble at 0°C. for 16 hrs.
Contents centri- fuged and ser- um separated and divided in two parts (table IV).	Ninhydrin test —	Contents centri- fuged and ser- um separated and divided in two parts (table IV).	Ninhydrin test —	Ninhydrin test +
A	B	C	D	E

Three c.c. of pregnant serum were placed in a centrifuge tube together with 1 gm. of boiled placenta protein. The contents of the tube were covered with a layer of toluol, stoppered with a cork, and put into the icebox (Table III-A). Parallel with this, 1.5 c.c. of the same serum with placental were placed in a dialysing thimble and suspended in a bottle with distilled water, as for a regular Abderhalden test, with the only difference that instead of placing it in the thermostat, it was put into the icebox (Table III-B). As

a control, an exact duplicate of these was put up, with male instead of pregnant serum (Table III-C and D). In addition to these another control was made by placing 5 c.c. of 1 per cent silk peptone in a dialysing thimble (Table III-E). At the end of sixteen hours the results tabulated above were obtained.

While the dialysate from the peptone gave a positive ninhydrin reaction (Table III-E) showing that dialysis was not arrested at 0°C., the Abderhalden test with both pregnant and normal sera gave negative results (Table III-B and D), which in view of the findings with the silk peptone meant that no dialysable substances were formed in the thimbles with pregnant as well as with male serum at 0°C.

TABLE IV.
The Appearance of Dialysable Substances in Pregnant Serum After the Removal of Placenta.

Serum A of table III		Thimble B of table III	Serum C of table III		Thimble D of table III
1.5 c.c. in thimble on ice for 16 hrs.	1.5 c.c. in thimble at 37° C. for 16 hrs.	Transferred to 37° C. for 16 hrs. in fresh distilled water.	1.5 c.c. in thimble on ice for 16 hrs.	1.5 c.c. in thimble at 37° C. for 16 hrs.	Transferred to 37° C. for 16 hrs. in fresh distilled water.
Ninhydrin test —	Ninhydrin test +	Ninhydrin test +	Ninhydrin test —	Ninhydrin test —	Ninhydrin test —
A'	A''	B	C'	C''	D

Having thus ascertained that the digestion did not occur on ice, both centrifuge tubes containing placenta with male and pregnant serum respectively (Table III-A and C) were removed and the contents of the tubes centrifuged at high speed. After ten minutes' centrifugation the serum was separated from the placenta in each tube and equally distributed into two thimbles each (Table IV-A' and A'', C' and C''). These were placed in bottles containing distilled water, the fluid inside and outside of the thimbles was covered with toluol, and one of each set of the bottles (Table IV-A' and C') placed in the icebox, the other (Table IV-A'' and C'') in the incubator. At the same time the thimbles containing a regular Abderhalden test were transferred to the thermostat (Table IV-B and D). After sixteen hours' incubation at 37° C. a ninhydrin reaction was made with each dialysate, and the results obtained were those tabulated above.

Thus it seems that by allowing the pregnant serum to combine with placenta at a low temperature (Table III-A and B) it was possible to resolve the Abderhalden test into two phases, indicating that it is not a simple, but a composite reaction. The experiment above shows that dialysable substances appear only in the second phase of the reaction, after the placenta has been removed (Table IV-A''). The fact, moreover, that their appearance followed the combination of pregnant serum and placenta only after such a serum separated from placenta, was incubated at 37°C., and that they did not appear at 0° (Table IV-A') confirm our prior conclusion that the dialysable substances during the second phase result from the *autodigestion of the serum and not from the digestion of the placenta.*

These findings are not only of theoretical importance, inasmuch as they furnish further proof of the similarity of the phenomenon of the Abderhalden with the immunity reaction, but are also of practical value. To those making routine examinations by the Abderhalden method, it is known that the blood of a patient taken under certain conditions, as when there is high temperature, pus

formation or recent ingestion of food may contain an amount of amino acid sufficient to mask the specific reaction.¹⁰² Whereas the last mentioned factor can be regulated with little inconvenience to the patient, blood being taken before breakfast, it is impossible to obviate the complications in the other cases.¹⁰³ In such instances, where the serum alone contains dialysable ninhydrin reacting substances the difference in the intensity of color reaction with ninhydrin of the control tube, and the tube containing placenta (or other substratum), as well as serum, furnishes the basis for diagnosis, according to Abderhalden.¹⁰⁴ The findings illustrated on the tables above suggested to us a procedure¹⁰⁵ which would allow the examination of any human or animal fresh serum taken at any time, no matter what the condition of the patient might be. This modified procedure is the following:

After remaining over night in contact with the suspected serum in the ice-box, the placenta (or other substratum, as the case may be) is centrifuged, washed with water to remove any serum that may stick to it, and placed in a new thimble with any serum known to be free from dialysable ninhydrin reacting substances. The best for this purpose is serum from a guinea-pig kept without food long enough (6-8 hours) to free its blood from dialysable reactive substances. It is necessary, of course, to take the additional precaution, in the diagnosis of pregnancy, of using male guinea-pig serum, since fresh serum from a pregnant guinea-pig gives a positive reaction with human placenta, and may thus cause grave error.

ANTITRYPTIC INDEX AS A METHOD OF REGISTERING THE ACTIVATION OF SERO-ENZYME

The method just described permits to avoid errors in diagnosis by the dialysis method without the recurrence to the method of Schlimpert which is inconvenient for several reasons discussed in one of the preceding paragraphs. However, the fact that in the modification offered by us the use of thimbles and ninhydrin still plays an essential part of the procedure, opens the method to the same criticism which we directed before against the original method. We tried therefore to find a method by which we could register the specific interaction between the immune serum and its corresponding substratum without recurrence to dialysis.

The conception of the mechanisms of the proteolysis taking place in the test, outlined by us previously in this paper, offers such a possibility. Namely, in our early experiments¹⁰⁶ we concluded that the combination between the antibodies of specific serum with the antigen (substratum) is accompanied by a physico-chemical change of the medium. This in turn causes the falling out or inactivation of antitrypsin of the serum, which originally prevented the activity of the normal proteolytic enzyme. The measurements taken by means of stalagmometer as well as refractometer leave no doubt as to the physical nature of

¹⁰²Abderhalden: Munch. Med. Woch., 1914, p. 401; also Lange: Berl. Klin. Woch., 1914, p. 785; and others.

¹⁰³For fuller discussion see one of the preceding parts of this paper. The presence of dialysable material in the fluid in which the specific ferments are looked for, is especially important in the examination of urine, in which there is always a certain amount of such substances present.

¹⁰⁴Abderhalden: Abwehrfermente, 4th Ed., p. 273.

¹⁰⁵Bronfenbrenner, Mitchell and Schlesinger: Bioch. Bull., 1914, iii, p. 386; also Bronfenbrenner, Schlesinger and Mitchell: Journ. of Amer. Med. Assn., 1915, p. 1268.

¹⁰⁶Bronfenbrenner: Proc. Soc. Exp. Biol. and Med., 1914, xii, p. 4.

the phenomena.¹⁰⁷ We showed definitely that this physical change of medium affects the activity of ferments of the serum through the inactivation of serum antitrypsin in two ways. On the one hand we succeeded in checking the appearance of dialysable substances in the Abderhalden test by means of increasing the amount of serum antitrypsin ^{108 109} (as suggested by the experiments of Schwartz¹¹⁰). On the other hand we actually measured the decrease of the antitryptic titer of the serum during the reaction. We found that the diminution of the antitryptic activity of the serum, as tested against trypsin solution, takes place in a specific manner, inasmuch as it occurs only in cases where the serum contains specific antibodies and is parallel with the intensity of the Abderhalden test; so that the estimation of antitrypsin in serum undergoing digestion may be used as a method of diagnosis parallel with, and complementary to, that of the Abderhalden test. Moreover, if the Abderhalden test is divided into two parts, as was shown before in this paper, over thirty per cent of the antitrypsin is removed during the first part of the reaction.¹¹¹

The procedure of this method is as follows:

The unknown serum is placed in a sterile centrifuge tube (1.5 c.c.) with a suitable amount of substratum (previously carefully standardized), stoppered with a cork and allowed to remain on ice for 18 hours (over night). At the same time the antitryptic titer of the serum is determined by any of the standard methods.¹¹² At the expiration of 18 hours the contents of the tube are centrifuged, serum separated, its antitryptic titer is again determined and the remainder of the serum is placed in the thermostat. It is obvious that parallel with this main test, one must conduct also two control tests (one with the known immune and the other with a known normal serum). If the unknown serum contained the antibodies in question one will notice a distinct drop in its antitryptic titer taking place during the sojourn of the serum on ice. Besides, such serum, when transferred to a thermostat (after separation from placenta) becomes turbid, whereas the serum, not containing specific antibodies, does not show either decrease in its antitryptic titer nor turbidity following the incubation in a thermostat.

The simplicity of this method is very apparent. In addition to the fact that such method eliminates the dialysis and subsequent ninhydrin test, both of which are known to be the cause of many discrepancies in the results obtained by the original Abderhalden method, such method as the one just described is convenient also because it allows to examine also the sera containing dialysing substances previous to their incubation with substratum.¹¹³

However, we do not wish to convey a false impression that this method is altogether as simple as just described. We wish to remind, that in such test as this, a very careful adjustment of the relative amounts of serum and substratum is most essential, as an excess of substratum may cause the diminution

¹⁰⁷These experiments are being reported in collaboration with Dr. M. Fleisher of St. Louis.

¹⁰⁸Bronfenbrenner: *Proc. Soc. Exp. Biol. and Med.*, 1914, xii, p. 6.

¹⁰⁹Bronfenbrenner: *Pennsylvania State Med. Journ.*, October, 1914.

¹¹⁰Schwartz: *Wien. Klin. Woch.*, 1909, p. 1151; also Jobling and Peterson: *Journ. Exp. Med.*, 1914, p. 239.

¹¹¹Bronfenbrenner, Mitchell and Titus: *Bioch. Bull.*, 1914, iv, p. 86.

¹¹²We used mostly the method of Brieger and Trebing (see *Berl. Klin. Woch.*, 1908, No. 22, No. 29, and No. 51).

¹¹³Bronfenbrenner, Andrews and Scott: *Journ. Amer. Med. Assn.*, 1915, p. 1306.

of antitrypsin in a normal serum at least as often as it does in the original Abderhalden test.

By performing the Abderhalden test in two phases, one can make two tests, using the same material. Namely, after having separated the serum from substratum following their sojourn on ice for 18 hours, one may take off a few drops of serum for the determination of antitryptic index and by placing the rest of the serum in a dialysing thimble, one can complete the Abderhalden test according to procedure described by us before.

THE ANAPHYLATOXIN FORMATION, AS DIAGNOSTIC TEST FOR SPECIFIC AUTODIGESTION OF THE SERUM

We have shown in preceding paragraphs that the combination of the immune serum with its corresponding substratum is followed by the autodigestion of the serum.¹¹⁴ We have observed that the split products of such autodigestion of serum are toxic to homologous animals and probably identical with so-called anaphylatoxin.¹¹⁵ The early observations in this direction suggested the possibility of using this specific formation of toxic products for detection of antibodies.¹¹⁶

The method was originally used by us for diagnosis of tuberculosis and consists in injecting into the skin of normal guinea-pigs 0.05 c.c. of a suitable mixture of the patient's serum and tuberculin upon which, in positive cases, there results within the next twenty-four to thirty-six hours, at the site of the inoculation, a reaction similar in aspect to a tuberculin reaction. Each series of guinea-pigs is accompanied by a control guinea-pig receiving intradermically the double dose of tuberculin alone. In addition to this, as a control for the serum, each guinea pig is injected, on the side opposite to that of the test injection, with double the amount of serum alone.

The results with the serum skin test (as we called this reaction) although quite uniform when originally applied to the diagnosis of experimental tuberculosis in guinea-pigs, were not quite as satisfactory when applied in the cases of human tuberculosis.¹¹⁷ As the formation of anaphylatoxin depends on the specific combination between antigen, antibody, and complement, and as the amount of circulating antibody varies very markedly in different tuberculous individuals, at first we were inclined to think that the failure to obtain more regular results with human sera was due to this difference in antibody contents in different cases. Our later studies on the mechanism of the formation of anaphylatoxin¹¹⁵ suggested however a more probable explanation of our failure to obtain uniform results with human material. As we have shown, the combination of antigen with its corresponding antibody results in autodigestion of the serum containing the antibody. The products of such autodigestion of the serum are however toxic only to the animals of the same species. Thus in the experiments reported above the anaphylatoxin produced as a result of auto-

¹¹⁴Bronfenbrenner: *Proc. Soc. Exp. Biol. and Med.*, 1914, xii, p. 7. These findings were recently confirmed by Eggstein: *Journ. Amer. Med. Assn.*, 1915, p. 735.

¹¹⁵Bronfenbrenner: *Pennsylvania State Med. Journ.*, October, 1914; also *Bioch. Bulletin*, 1915, iv, p. 87; also *Journ. Exp. Med.*, May, 1915, xxi, p. 480. These findings are corroborated by Jobling, Peterson, and Eggstein in the current number of the *Journ. Exp. Med.*, 1915, xxii, 401.

¹¹⁶Bronfenbrenner: *Proc. Soc. Exp. Biol. and Med.*, 1914, xii, p. 90; also *Science*, 1914, p. 803.

¹¹⁷Bronfenbrenner: *Trans. Nat. Assn. Study and Prev. of Tuberc.*, May, 1914.

digestion of human serum was not uniformly toxic to guinea-pigs. We, therefore, changed the method in the following manner.¹¹⁸

The serum of the patient is injected intraperitoneally into a normal guinea-pig thus transferring upon the guinea-pig the specific properties of the patient's serum. Twenty-four hours later this guinea-pig is bled, its serum is placed upon ice in a test tube with a suitable amount of substratum (placenta, tuberculine, B. E., tumor tissue, etc., as the case may be). Next day (twelve to eighteen hours later) the serum is centrifuged off and placed in the incubator for from twelve to eighteen hours. During this incubation the serum undergoes autodigestion and toxic split products of the serum are formed, as we have shown before.¹¹⁹

At this time 0.05 c.c. of such autodigested guinea-pig serum is injected into the skin of a normal guinea-pig on a spot previously shaven. From twelve to twenty-four hours later one observes a very distinct skin reaction on the place of the injection if the serum of the patient used in the test contained specific antibodies. Instead of injecting the autodigested serum into the skin one can inject 0.5 c.c. of this serum into the heart or in the vein of a normal guinea-pig in which case the guinea-pig will die with symptoms of acute anaphylactic shock if the serum was specific.

If the serum of the patient did not contain specific antibodies, the serum of a guinea-pig (which received such a normal serum intraperitoneally) may be injected into the veins of normal guinea-pigs in the quantity of 5 c.c. and more, without causing any symptoms.¹²⁰

Although by these methods one avoids the complications incumbent upon the possible presence of dialysable substances in the serum of the patient as well as the possible errors connected with the use of thimbles and ninhydrin, it is necessary to remember that these tests depend on the autodigestion of the serum in the same degree as the original Abderhalden test, in the serum-skin reaction, for instance, the autodigestion of the serum may take place as a result of specific as well as nonspecific action of substratum upon serum. The test may be reliable therefore only if the quantity of substratum used is such that it is unable to cause autodigestion of the serum by merely mechanical adsorption.

The modifications we offer, therefore, do not improve the specificity or the sensitiveness of the Abderhalden reaction since they depend upon the same mechanism, but by using these methods we avoid the additional difficulties and discrepancies in results due to the complications in the reading of the results of the reaction, by the original method of Abderhalden.

Theoretically the methods suggested above are of interest because they substantiate our assumptions that the serum ferments are not specific, that the dialysable substances originate not from the substratum, but from the serum, that

¹¹⁸Bronfenbrenner: Proc. Soc. Exp. Biol. and Med., 1914, xii, p. 48.

¹¹⁹Bronfenbrenner: Journ. Exper. Med., 1915, xxi, p. 480.

¹²⁰The specificity of this reaction has not been tested out in sufficient number of cases to judge of its merits as compared with the Abderhalden reaction. As both tests depend on the autodigestion of the serum, which takes place as a result of combination of antibody, with the substratum, one should expect the two reactions to give identical results. Due to the fact, however, that the readings in Abderhalden Reaction may be influenced by imperfection of thimbles or by the delicacy of ninhydrin test, the anaphylatoxin test seems to offer a more reliable indicator for the specific autodigestion of the immune serum. Kolmer and Williams, who recently used this test in diagnosis of pregnancy obtained good results even although they used our method in its old imperfect form. (Kolmer and Williams: Am. Journ. Obst. and Dis. of Women and Child., July, 1915.)

in so far as the test is specific, its specificity depends on the presence in the blood of substances identical with the antibodies, and not specific ferments.

CONCLUSIONS

The critical analysis of the Abderhalden reaction as a diagnostic method brings us to the following conclusions:

If in performing the test one follows all the precautions prescribed for this method, if one in addition is able to control every step as the necessity arises, even beyond the prescribed procedure, one may obtain very satisfactory results in a number of cases.

There will be certain cases, in which the results obtained are not correct, but a competent serologist should be able to discard such findings, guided by his thorough control of numerous factors in the test. In its present form, therefore, the test undoubtedly requires that the men who perform it be thoroughly trained in serology as well as in chemistry of enzyme, if possible.

This requirement, however, does not justify Abderhalden in saying that the men who dissented with him as to the value of his test were not qualified to form their opinion on account of lack of experience. On the contrary, in going over the tremendous number of publications on the subject, one is impressed by the fact that a great majority of men who (if we take Abderhalden's view on the subject) were skillful enough to perform the test "properly" and obtained results satisfactory to Abderhalden—are men who never did any serological work before, or did very little. And on the other hand we see a number of prominent serologists who say that the test may show specific results, but the fact that digestion can be induced in any serum, speaks against the specificity of the "Abwehrfermente."

A certain number of mistaken diagnoses have been made, of course, by the men who were not competent to even properly control their tests, and it is in order to eliminate the possibility of these mistakes that a number of improvements were recommended by different workers. Some of the modifications offered simply remove certain of the undesirable elements in the technic of the test, but do not change the essentials of the reaction. Other modifications are based on an entirely different conception of the phenomena from that offered by Abderhalden, and do away practically with the entire original procedure. But even these improved methods it seems will not save an ordinary laboratory worker from errors mainly, because the most important element, which determines the specificity of reaction—the easy control of quantitative relation between the substratum and serum—is still not at hand. It is true, that by exercising special care one could in most cases approach the biological truth using the specific activation of sero-enzyme in vitro as indicator of specific processes in the body, but the fact alone that such test takes considerable time for each individual case (if the substratum is properly standardized), and cannot be entrusted to a laboratory technician, makes it impractical for the diagnostic laboratory.

The test still remains comparatively useful in special cases, when the results obtained may justify the expenditure of time of a highly trained worker, but even there, I feel, though often correct, the results should be taken with

reserve until, as I said before, the substratum is obtained in such a form, which would permit its easy standardization.

The amount of substratum used in the test cannot be uniform in all cases because not only, as I have stated, different immune sera react with various amounts of substratum according to the amount of specific antibody present, but also in case of normal sera, the amounts of substratum capable to induce autodigestion vary with different sera. Some of our most recent investigations conducted in collaboration with Dr. M. Fleisher indicate that usual quantitative variations in the relative amounts of different colloidal constituents of normal blood may influence the rate of specific as well as nonspecific colloidal reactions upon which the autodigestion of serum *in vitro* depends.

It is essential, therefore, to obtain the substratum in some such form as a uniform emulsion or even solution in order to allow a more intimate and a more general interaction between substratum and specific elements of the serum and at the same time to permit a most careful titration of the substratum, so as to preclude the possibility of a non-specific adsorption by it of the colloidal serum constituents.

As it stands at present, the Abderhalden reaction has only a scientific interest, and in that its main value is in the fact that it stimulated the studying of the fermentative activities of the body fluids and especially of the blood.

Although these studies tend more and more to deny the basic discovery of Abderhalden, namely that the body is endowed with the ability to respond with the production of specific ferments upon the parenteral introduction of foreign substances, it is evident that the role of normal nonspecific ferments of the blood received much more attention in connection with the phenomena of immunity and with parental digestion in general, than it was given before.

PROTEIN METABOLISM

BY J. J. R. MACLEOD, M.B., CLEVELAND, OHIO.

AS an outcome of the work of numerous investigators, the exact chemical constitution of most of the simpler molecules which are linked together to compose the much larger one of protein itself has become known, and a little has been gleaned regarding the manner by which those molecules are bound together. To grasp the main principles regulating the highly complex structure of protein it is of value to think of its molecule as a finished building, and of the degradation products (the amino acids, etc.) as building materials. Into the structure of such a building, materials might enter which are the same as those used for other buildings, and yet no two of the buildings be exactly alike. According to this view the protein molecule is a very complicated structure built up from simpler molecules (the building stones), which, although they are of the same general chemical nature, are never used in exactly the same proportions, or joined together in the same manner in different proteins.

This conception of the structure of protein is of especial importance not

only in the study of protein metabolism, for which we are to use it here, but also in enabling us to grasp the almost unthinkable number of varieties in which protein exists, without there being, in many cases, any outward chemical reaction or physical property by which one protein can be distinguished from another. It is particularly important to realize, however, that differences in structure that are too slight to be recognizable by ordinary chemical means may become very apparent when the biological method is used, that is to say, when we proceed to observe the behavior of an animal into whose blood some of the protein is directly injected. As is well known, symptoms of varying severity develop; from the almost instantaneous death produced by snake venom to the slowly developing anaphylactic reactions which follow the injection into the blood of many proteins that are chemically indistinguishable from those of the blood itself. When the protein is taken by mouth these poisonous reactions do not supervene—even snake venom is harmless when swallowed—nor is it possible, during digestion of a protein meal, to detect food proteins in the blood by means of the precipitin reactions. Another very significant point in this connection is that no proteolytic enzymes, capable of digesting food-proteins, are present in normal blood, but they immediately appear following injection of food-protein. That the enzymes are absent in normal blood must, therefore, indicate that food proteins cannot be absorbed as such.

What causes the difference between the *parenteral* and the *alimentary* absorption of proteins? This evidently is a fundamental problem not of biochemistry alone, but of the closely related science of immunology as well. The answer is that protein is absorbed only after being completely broken down into the amino acids, which are then rebuilt into the proteins of the organism. This view, however, did not for many years receive any direct experimental support, although many facts served to offer circumstantial evidence in favor of it. Among these may be mentioned the discovery of Buglia¹ that the very slow intravenous injection of completely digested flesh did not produce, on the part of the body, any of the reactions which we have seen protein itself produces, thus indicating that perfect assimilation had occurred. Other investigators succeeded, either in separating amino acids from the blood when very large and unusual quantities were directly placed in the intestine (Abderhalden, Guyon, and London) or in demonstrating that the non-protein nitrogen present in the blood became increased over its amount during starvation, not only when amino acids were placed in the intestine, but also during the digestion of protein food. Such results could, however, scarcely be given much weight in deciding whether, under *normal* conditions, protein is absorbed as its degradation products, for it could be argued that, just as egg-albumin is absorbed unchanged into the blood when very large amounts are present in the intestine, so also might amino acids. It had to be shown that such substances are actually present in the blood when digestion of protein is proceeding under normal conditions, and this, no one could succeed in doing.

To account for the indisputable disappearance of amino acids from the intestine during protein digestion, without any trace of them detectable in the blood, two views were current for many years. One of these was that the

¹Buglia: Ztschr. f. Biologie, lviii, p. 162 (1912).

amino acids become deaminated (NH_2 group split off as NH_3) by the intestinal epithelium, and the other that these cells are endowed with the power of recombining the amino acids into protein, which then passes into the blood.

Justification for the deaminization hypothesis seemed to be obtained by the observation that there is more free ammonia in the blood of the portal vein than in that of the systemic circulation. The falsity of this argument was definitely shown by Folin and Denis,² who found, by means of delicate quantitative methods for the estimation of ammonia and urea, the principle of which will be described later, that the amount of neither of these substances became increased in the portal blood during absorption of amino acids from a loop of intestine. They made the further important discovery that the ammonia in the portal blood is really very small in amount and represents the absorption of ammonia as such from the intestinal lumen, where it is produced, chiefly by putrefactive bacteria.

Nor could any evidence be obtained in favor of the hypothesis that the absorbed amino acids become built up into blood proteins; it was an hypothesis based entirely on negative findings, and had to be dropped when discovery was made of the actual presence of amino acids in the blood.

The above general statement of the development of our knowledge of the history of protein in the animal organism brings us to the problem as it stands at the present day, and it becomes of interest to examine into the methods that are being employed by biochemists in its further elucidation. It may be stated that these methods are three in number, viz.:

1. Separation of the amino acids from the blood by dialysis (Abel, Abderhalden).
2. Comparison of the amount of non-protein nitrogen of the blood and tissues before and during food-absorption (Folin, etc.).
3. Comparison of the amount of amino nitrogen in the blood and tissues before and during food-absorption (Van Slyke, etc.).

We shall endeavor to point out the most important discoveries brought to light by these methods.

I. The Dialyser Method: Abel and Turner³ were the first to succeed in causing the diffusible amino acids to leave the blood by dialysis in sufficient amounts for proper chemical identification. Their success, where others had failed, was due to the use of a so-called vivi-diffusion apparatus, which consists of a long tube of celloidin connected at one end with an artery and at the other with a vein of an anæsthetized animal. The tube is immersed in a saline solution approximating in composition to the salt content of the serum of the animal. All diffusible constituents of the blood plasma accumulate in the saline solution, or if it be desired to prevent any one of them from diffusing, it is only necessary to add that particular substance to the saline in such amount as will make its concentration in plasma and saline alike. In some ways, it will be seen, the apparatus may be considered as an "artificial kidney." Its possible clinical application, for the purpose of removing poisons from the blood, is being investigated. With the apparatus it has been possible to isolate several of the amino acids and other ammonia-yielding substances from blood. Thus

²Folin and Denis: *Jour. Biol. Chem.*, xi, p. 161 (1912).

³Abel and Turner: *Journal of Pharmacology and Experimental Therapeutics*, v, pp. 275 and 625 (1913).

alanine and valine were obtained in crystalline form, and histidine and creatine could be shown to be present by their reactions. All of the amino substances do not, however, dialyse, for Rohde, working with Abel, has shown that there are certain of them that do not diffuse and are further characterized by the fact that they do not readily give up their ammonia on the addition of sodium carbonate, which the diffusible substances do.⁴

II. Comparison of the Amount of Non-protein Nitrogen in the Blood and Tissues: As explained above, it was mainly by the use of this method that Folin and his pupils were able to show that neither a deaminating nor a synthesizing function resides in the intestinal epithelium. Although his methods do not permit us to state that amino acids *as such* are absorbed into the blood, the results which he obtained can not be interpreted on any other basis. The substances estimated in the blood are the non-protein nitrogen, the ammonia and the urea. The non-protein nitrogen and the urea are converted into ammonia, which, along with the ammonia originally present, is then determined by adding sodium carbonate and bubbling air through the mixture and then through a weak acid to catch the liberated ammonia. Nessler's reagent for ammonia is then added to this flask and the intensity of the orange color which develops is compared in a Duboscq colorimeter with that given by a standardized ammonia solution mixed with the reagent. Nessler's test is an extremely sensitive one, being used in water analysis, so that with due care very accurate estimations can be made, even on a few c.c. of blood.

For non-protein nitrogen, the proteins in an accurately measured quantity of blood are precipitated by methyl alcohol and zinc chloride, and an aliquot portion of the filtrate, after being heated to get rid of the alcohol, is digested with concentrated sulphuric acid in the presence of copper and potassium sulphates until all of the organic matter has become oxidized (Kjeldahl's process). The clear incineration residue contains all of the nitrogen, that was present in the original material, as ammonium sulphate. It is diluted with water and transferred to a cylinder in which, after being mixed with alkali, the ammonia is driven out of it by an air stream and estimated as above described.

For ammonia, a larger amount of blood is taken and, after dilution, is mixed with sodium chloride and sodium carbonate and the ammonia driven off by the air stream.

For urea, Folin's method, which was somewhat difficult, has come to be superseded by that of E. K. Marshall, Jr., which is extremely simple, and depends on the conversion of the urea into ammonium carbonate by means of the "urease" of the soy bean. The ammonia is then estimated by Folin's method. By subtracting the ammonia found present in another sample of the same blood from that found after the action of urease, the ammonia derived from urea is determined. Certain drug manufacturers produce "urease" in the form of a dry powder which is extremely active and can entirely decompose all the urea in blood or urine in a few minutes.

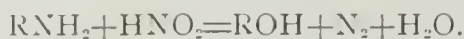
It will be noticed that the above is no direct method for estimating amino acids; which, however, along with certain other ammonia-yielding substances,

⁴Cf. Abel: The Mellon Lecture; Science, xlii, p. 135 (1915).

such as creatinine, uric acid, etc., make up the remainder, after subtracting the nitrogen of urea and ammonia from the total non-protein nitrogen.

We shall not go into the actual experiments which Folin and his pupils have performed by the use of these methods, because the conclusions are pretty much the same as those reached by Van Slyke, etc., which are given below. In a future review we hope to show how Folin's methods have been used to investigate the retention of nitrogenous substances in nephritis, etc.⁵

III. Comparison of the Amount of Amino Nitrogen in the Blood and Tissues Before and During Food-Absorption: In a series of papers appearing in the *Journal of Biological Chemistry*, D. D. Van Slyke, along with P. A. Levene and G. M. Meyer, has described in detail a method for estimating amino nitrogen in blood, in tissues, and in urine.⁶ The method depends on the well-known reaction represented in the equation:



The rate with which different substances containing the amino group evolve their nitrogen is not the same; amino acids, most proteins and hydrolyzed (digested) proteins become decomposed, with vigorous shaking, in five minutes; native proteins (i. e., those coagulable by heat) require longer, because of the formation of insoluble coagula which mechanically retain the nitrogen; amino purines (guanine and adenine) require more than five minutes. Ammonium salts are also decomposed by the reagent, but at a much slower rate; to avoid confusion due to the presence of ammonia, the fluid to be tested can be first of all aerated with $\text{Ca}(\text{OH})_2$ and the liberated ammonium driven off. Urea, as is well known, also liberates its nitrogen with nitrous acid, but it does so much more slowly than the amino acids; thus, in the time that amino acids yield 100 per cent of their available nitrogen, urea yields only 3 per cent. When only small traces of urea are present, as in blood, the error which they would introduce can be eliminated most simply by first of all measuring the nitrogen eliminated in 2 to 4 minutes (according to temperature) and then continuing the decomposition for the same number of minutes and subtracting the nitrogen evolved during the second period from that of the first. The urea is decomposed at a uniform rate, which is indicated by the second analysis.

When the urea is large in proportion to the amino acids, as in urine, it can be removed by means of urease, an enzyme present in the soy bean and which quickly decomposes urea into ammonium carbonate. The ammonium thus produced, along with that originally present in the urine, can then be removed by shaking the urine with lime (calcium hydrate), filtering, and evaporating to dryness on a water bath.

The apparatus employed for decomposing the substance and collecting and measuring the evolved nitrogen consists essentially of a mixing bulb connected below through stop cocks with two small burettes, one containing a solution of

⁵The important papers are as follows:

Folin and Denis: *The Journal of Biological Chemistry*, xi, (1912), p. 87 and p. 493; xii (1912), p. 141 and p. 253.

Folin and Lyman: *ibid.*, xii (1912), p. 259.

Marshall: *ibid.*, xv (1913), p. 487.

⁶The fundamental papers are as follows: Donald D. Van Slyke: *Journal of Biological Chemistry*, Vol. ix, p. 185 (1911); Vol. xii, p. 275 (1912); Vol. xii, p. 301 (1912 (with P. A. Levene); Vol. xii, p. 399 (with G. M. Meyer); Vol. xiii, p. 121 (1913); *ibid.*, p. 125 (urine); *ibid.*, p. 187 (tissues).

sodium nitrite and glacial acetic acid, the other a solution of the substance to be investigated. The upper end of the bulb is connected by a three-way cock with a graduated gas burette and a Hempel bulb containing potassium permanganate solution. By allowing some nitrite and acid solution into it and shaking, the mixing bulb is first of all filled to a certain mark with nitric oxide gas. A measured quantity of the amino solution is then allowed to run into and mix with the nitrite which remains, the apparatus is shaken for five minutes at 15-20 degrees C., and the evolved nitrogen and nitric oxide are driven over into the permanganate, which absorbs the nitric oxide, leaving the nitrogen, which is then measured in the burette. To ensure thorough liberation of the nitrogen, the apparatus can be mechanically shaken, and it is so assembled that several consecutive analyses can be made without separating any of the parts. A small or micro apparatus has been devised, the accuracy of which is such that it entirely suffices for all physiological and clinical purposes (e. g., it requires only 0.5 mgm. of amino nitrogen for an analysis accurate to within 1 per cent).

In estimating the amino substances in blood, the proteins are first of all removed by alcohol and the alcohol along with any free ammonia, subsequently removed by evaporating in faintly alkaline reaction. To estimate the amino substances in tissues, the weighed amount of these is cut up and thoroughly extracted by boiling with weak acetic acid, the extract treated with alcohol to remove traces of protein, then concentrated *in vacuo* to suitable bulk, and the amino nitrogen determined by the usual method.

The results which have been obtained by Van Slyke and his collaborators are of fundamental importance. We shall state them briefly and then proceed to show how they may be pieced together so as to furnish us with a more or less complete history of protein in the organism after it has been absorbed from the intestine.

1. The blood of fasting animals (dogs) contains from 3 to 5 milligrams of amino nitrogen per 100 c.c. of blood.

2. In blood removed five minutes after 12 grammes of the well-known amino acid, alanin (amino propionic acid) had been injected intravenously (the injection occupying 13 minutes) it was found that only 1.5 gms. remained in the blood, and after 35 minutes, only 0.5 gm.; 1.5 gm. of the injected alanin was excreted by the urine leaving therefore some 10 grammes, which must have disappeared somewhere in the organism.

3. Absorption of 10 gr. alanin from the small intestine increased the amino nitrogen of the mesenteric blood from 3.7 to 6.3 mgms. per cent.

4. During the normal digestion of meat the amino content of the blood undergoes a marked increase—doubled or more—compared with its value before feeding.

5. This increase affects the blood of the femoral artery almost as much as that of the mesenteric vein.⁷

6. The amino bodies which disappear from the blood are merely absorbed by the tissues without undergoing any immediate chemical change

7. In the case of the muscles at least, there is a fairly definite saturation

⁷Van Slyke and G. M. Meyer: Journ. Biol. Chem., xii, p. 349. (First five results.)

point above which it is impossible to force the amino-acid saturation. This is between 75 and 80 mgm. per cent.

8. The capacity of the intestinal organs is more elastic; thus, the amino nitrogen of the liver may be raised to 125-150 mg. per cent.

9. Although the tissue absorption of amino acids is extremely rapid, it never proceeds to such a point that the blood becomes entirely free of them. Thus the blood contains from 3 to 8 mgs. of amino nitrogen per cent even after a fast of several days' duration. A certain equilibrium must therefore exist between the amino content of blood and tissues, the concentration in the tissues being from 5 to 10 times more than in the blood.⁸

10. In one-half hour, and again in several hours, after the slow intravenous injection (occupying 1 to 1½ hours) of non-toxic amounts of glycocoll (amino acetic acid) or of mixtures of amino bodies obtained by the hydrolysis of casein or complete artificial digestion of meat, portions of various tissues were removed and the percentage amounts of amino nitrogen determined. It was shown that absorbed amino acids disappear rapidly within 2 or 3 hours from the liver, but that meanwhile none has disappeared from the muscles. The other organs seem to behave more like the muscles than the liver.

11. The disappearance of amino bodies from the liver is accompanied by an increase in the urea of the blood.⁹

12. Free amino nitrogen is present in the tissues of starving animals in as great, if not greater, concentration than in those of fed animals.

On the basis of the evidence furnished by these and previous results—particularly Folin's—we may sum up the present-day knowledge regarding the essential Physiology of Proteins in the animal body as follows:

By the agency of the digestive juices the large and complex protein molecule is disintegrated into its constituent amino acids (i. e., building stones), a few probably remaining attached to one another as polypeptids. Some of these amino acids are further destroyed by the intestinal bacteria, but the great majority are absorbed unchanged into the blood of the portal system, which carries them past the liver into the systemic circulation without any recognizable diminution in concentration. They do not remain for long in the blood, however, because the tissues greedily absorb them, or if the blood is suddenly loaded with very large quantities, some may be excreted as such into the urine. This absorbing power is very considerable in the muscles, but it is still more marked in the liver, although even this organ does not absorb the amino acids quickly enough to make it possible to detect any difference between the percentage of amino nitrogen in portal and systemic blood. The tissues do not continue to absorb the amino acids from the blood until this is free of them, but only to a certain level below which, even by prolonged starvation, it is impossible to lower the amino acid content. Nor is it possible, by the same means, to cause the tissues to lose all their amino acids, thus indicating that a certain equilibrium must exist between the amino nitrogen of tissues and blood. These facts further indicate that there can be an immediate chemical change in the ab-

⁸Van Slyke and G. M. Meyer: *Journ. Biol. Chem.*, xvi, p. 197. (6th to 9th results.)

⁹Van Slyke and G. M. Meyer: *Journ. Biol. Chem.*, xvi, p. 231.

sorbed amino acids, and they explain the transfer of protein "building stones" which it has been known for long must occur from organ to organ during starvation and from maternal organs to foetus.

The amino acids at first deposited in the tissues must later become built into tissue protein, but since proteins vary with regard to the exact amino acids (building stones) used in their construction, considerable quantities of those substances cannot be required.

These "rejected building stones" are again taken up by the blood, and if not required by some other variety of protein in the organism, are probably carried to the liver—or to other organs as well (Folin)—where the nitrogenous portion of them is converted into urea. Since amino acids are present in the blood even during prolonged starvation, a constant migration of amino acids must also be going on between tissue and tissue; these amino acids must be produced by disintegration processes of the tissue protein, a physiological autolysis. Some of the migrating "useful" building stones must add themselves to the rejected ones and become converted to ammonia and urea in the liver. The liver, by constantly metabolizing the amino acids which it absorbs, has a great capacity for removing these substances from the blood. This supports the long-contended view that the liver is especially responsible for the catabolism of those protein digestion products not utilized for tissue construction. There is experimental evidence to show that when the above agencies, including the excretory power of the kidneys, for preventing undue accumulation of protein digestion products are overtaxed (e. g., as in disease of the liver), death may result. We shall, however, reserve for a future article a further consideration of these pathological chemical findings.

FURTHER STUDIES ON THE TREATMENT OF SYPHILIS OF THE NERVOUS SYSTEM BY INTRADURAL INJECTIONS (OLD SALVARSAN)

BY UDO J. WILE, M.D., ANN ARBOR, MICH.

IN a previous report¹ I detailed a method of treating syphilis of the cerebrospinal axis by the intradural injections of neosalvarsan, at first suggested by Ravaut and subsequently modified by me.

My results on some fifteen cases thus treated were reported and in the main, although there were some discouraging features, they led me to the firm conviction, expressed in that article, that the future therapy of syphilis of the cerebrospinal axis lay in the direct treatment of the nervous system itself. My conviction then was, and now is that such therapeutic measures would embody the use of salvarsan or an elaborated salvarsan derivative, which could be used in sufficiently large doses without injuring the nerve tissue, rather than the employment of the salvarsanized serum, as employed in the technique of Swift and Ellis.

¹Wile: The Technic of Intraspinal Injection of Neosalvarsan in Syphilis of the Nervous System. Jour. A. M. A., Apr. 11, 1914, p. 1165; *ibid*, July 11, 1914, pp. 136-141.

Following my first communication on this subject, numerous other writers have reported their results with neosalvarsan, injected directly in the cerebrospinal canal, and in the main the results have been discouraging. Such untoward accidents as complete paraplegia, permanent paralysis of the bladder and rectum and severe neuralgic pain, were found to be common complications of the treatment.

I believe that such complications, which were described as occurring in three of my fifteen cases, were not so much due to the toxic nature of the drug itself, as to the relatively large dosage employed.

During the past year I have modified my technique materially; first, by greatly diminishing the dosage of the injected material, and, secondly, by substituting for neosalvarsan, old salvarsan. I have now employed this method in fifteen cases of cerebrospinal syphilis of various types and have noted very striking improvement in the majority of these cases and absolutely no untoward results in any. The technique employed, a simplification of that described by Ogilvie, is as follows:

The patient is punctured in the usual Quinke position, lying on his left side. After the canal has been successfully entered the barrel of a 40 c.c. Luer Syringe is attached to the needle by means of rubber tubing about eight inches long. The cylinder barrel is then lowered below the level of the point of puncture and the fluid is collected until about 10 c.c. have flowed back. The salvarsan is a freshly prepared solution of one decigram to 30 c.c. of water. Of this solution an amount corresponding to one-third of a milligram (approximately two minims) is now carefully dropped into the fluid by means of a specially graduated syringe, the assistant in the meantime holding the barrel containing the fluid. The two drops are carefully stirred into the cerebrospinal fluid by means of a sterile glass rod. The syringe barrel is then raised above the level of the puncture and the salvarsanized cerebrospinal fluid is allowed to run back into the canal by gravity.

The patient is then placed on his back and the bed elevated from six to twelve hours and the patient kept in bed for forty-eight hours, after which he is permitted to get up. Strict surgical technique is observed throughout the procedure.

In the cases thus treated and only in those having tabes, a slight amount of darting pain was noted for a few hours subsequent to the injection. This invariably disappeared within twenty-four hours and was the only untoward symptom noted. Among the fifteen cases treated were two of very desperate nature, one of meningo-myelo encephalitis (see Case 3), and another of hemiplegia and basal meningitis (see Case 9), in both of which active general treatment had failed to bring about any improvement and it seemed as though both cases might succumb. As criteria of improvement were noted not only the subjective symptoms but more particularly the objective findings.

On account of the marked tendency of paretic patients to remit, and on account of the tendency of such patients to be euphoric, no straight cases of paresis were treated in this fashion. I believe, furthermore, that paretics offer the least possible chances of improvement. Four tabo-paretics, however, were treated for the tabetic manifestations. They represent the greatest source of error on the part of the observer in reporting supposed subjective improvement.

A brief summary of the cases thus treated follows:

Case 1. Tabes dorsalis, age 31. Syphilis contracted twelve years previously. Primary optic atrophy, Argyll-Robertson pupil, lost Achilles and knee jerks, external rectus palsy, marked sensory disturbances in both feet, severe tabetic pains, difficulty in urination and defecation, gastric crises. Examination of the cerebrospinal fluid showed 90 cells, Nonne Apelt +++, Albumen +++, Wassermann on the fluid +++, Wassermann on the blood +++++.

April 13, 1915. Intradural injection one-third of a milligram of Old Salvarsan, followed the next day by shooting pains in the instep.

April 28, 1915. The patient returned subjectively improved, no more crises, walks better, tabetic pains in the legs still present, but much less severe in character. Examination of the cerebrospinal fluid showed 30 cells, Nonne Apelt positive in a dilution of one to one only; Wassermann on the cerebrospinal fluid still positive. Patient passed from observation, no further report.

Case 2. Tabes dorsalis, age 28. Syphilis contracted seven years ago. Sluggish pupils, lost tendon reflexes, marked Romberg, neuro-retinitis. Examination of the cerebrospinal fluid showed 17 cells, Nonne Apelt and Albumen positive. Wassermann on the cerebrospinal fluid is positive.

Jan. 18, 1915. Intradural injection one-third of a milligram of Old Salvarsan followed by some pain in the legs for six hours, subsequently felt better.

Feb. 24, 1915. Patient returned and walks better, says he feels as well as he ever did, has no pains, neuro-retinitis less marked, cerebrospinal fluid showed 10 cells, otherwise the same as before.

Case 3. Meningo-myelo encephalitis, age 43. No history of syphilis. Severe headaches for the past year, loss of memory, failing vision, difficulty in walking, neuro-retinitis with hemorrhage, three to four diopters of choked disc in both eyes. Acoustic examination shows loss of bone conduction.

March 26, 1915. Examination of the cerebrospinal fluid shows 240 cells. Nonne Apelt +++++, Albumen +++++, Wassermann on the cerebrospinal fluid +++++, Blood Wassermann plus-minus. This patient received three intradural injections at weekly intervals of one-third of a milligram Old Salvarsan.

April 12, 1915. Examination of the cerebrospinal fluid shows 200 cells. Nonne Apelt ++, Albumen and Wassermann still positive.

June 19, 1915. Examination of the cerebrospinal fluid shows 70 cells. Nonne Apelt and Albumen markedly diminished, Wassermann still positive.

Following the second injection almost complete clearing up of the choked disc, which was followed by complete recovery of the visual field, marked improvement in station. The headaches disappeared entirely and the memory is better. Patient was able to return to his work. The patient, previous to his injections, besides suffering from severe pain in the head, had a staggering gait and a markedly blurred vision.

July 23, 1915. Patient returns in every way improved. The spinal fluid examination shows 30 cells, but with the other findings in the cerebrospinal fluid unchanged. At the present writing the patient is actively at work. He writes that his eye-sight is as good as ever and that he continues to improve in general condition each day. The striking feature of this case is marked subjective improvement, parallel with striking objective improvement in the neurological findings as well as in the spinal fluid.

Case 4. Tabes dorsalis, age 37. No history of syphilis. Argyll-Robertson pupil, marked ataxia of the feet and hands, lost knee and Achilles jerks, marked Romberg and marked sensory disturbances. Ophthalmoscopic examination showed a periarteritis. Examination of the cerebrospinal fluid was positive throughout. Patient was given one intradural injection of Old Salvarsan, one-third of a milligram on June 7, 1915. He has not yet reported for re-examination, but before his temporary discharge from the hospital he was freer from pain than he had been for some time previously, otherwise no marked change. He has not been heard from since.

Case 5. Meningo-myelo encephalitis, age 40. History of syphilis fifteen years ago. Anesthesia and analgesia of the fingers of the right hand, increased knee and Achilles jerks, unequal pupils, weakness of the left internal rectus, severe pain in the legs and chest, difficulty in walking. Ophthalmoscopic examination; O. D. beginning optic atrophy and sclerosis of the vessels. Examination of the cerebrospinal fluid showed 80 cells and otherwise positive throughout. Patient received three injections of one-third of a milligram of Old Salvarsan intradurally at weekly intervals. Examination of the cerebrospinal fluid subsequent to his second injection showed 12 cells and markedly decreased other findings in the fluid. Following his third injection and subsequent observation of this patient over eight months, says he feels much better, is able to walk better; the ataxia is, however, neither better nor worse.

Case 6. Tabo-paresis, age 43. Syphilis twenty years previously. Pain in the stomach, dizziness, failure of memory, paralysis of the right leg seven years previously. Ophthalmoscopic examination: Old iritis and arteriosclerosis. Marked speech defect and Argyll-Robertson pupil and marked Romberg. Examination of the cerebrospinal fluid showed 45 cells Nonne Apelt +++++, Wassermann on the cerebrospinal fluid is++++. This patient received two doses of Old Salvarsan of one-third of a milligram intradurally at weekly intervals. Following his second injection the cerebrospinal fluid showed 25 cells and markedly decreased findings in the organic solids. The Wassermann is still +++++ positive.

Case 7. Tabo-paresis, age 46. Syphilis thirteen years ago. Lost knee and Achilles jerks, marked incoördination. Romberg is positive, slight incontinence, marked speech defect, dizziness and failure of memory. Paralysis in one leg seven years ago. Ophthalmoscopic examination: Old iritis and arteriosclerosis. Examination of the cerebrospinal fluid showed 20 cells, Albumen slightly increased, Nonne Apelt and Wassermann positive. Patient received two intradural injections of one-third of a milligram of Old Salvarsan at weekly intervals. He is still under our care and is to receive further treatment, says he feels much improved, his memory seems better, walks better and has no headaches, tires less easily and has now no incontinence.

Case 8. Tabo-paresis, age 52. Syphilis denied. Lost knee and Achilles jerk on the left side, nystagmus, diminished knee jerk on the right side, incontinence, memory and speech defect, staggers in the dark, pupils are unequal. Ophthalmoscopic examination shows edema of the nerve head. Examination of the cerebrospinal fluid shows 15 cells, Nonne Apelt and Albumen slightly increased. Wassermann on the cerebrospinal fluid and blood both +++++ positive.

December 7, 1914. Patient received one-third of a milligram of Old Sal-

varsan intradurally. Several weeks following his injection the patient reports that he feels much better, has less trouble in holding and starting the flow of urine, otherwise no marked changes.

Case 9. Cerebral arteritis, hemorrhage and basal meningitis, age 20. Syphilis six months ago. Patient was brought to the hospital unconscious, markedly cyanosed, involuntary movements of the whole right side. Hemiplegic, irrational and at times stuporous. Ophthalmoscopic examination: marked neuroretinitis and edema of the nerve head. Examination of the cerebrospinal fluid previous to treatment showed 600 cells and four plus throughout. Patient received three injections of one-third of a milligram of Old Salvarsan intradurally. Following the first injection, marked cessation of the involuntary spastic contractions, the cyanosis improved and the mental condition began to clear up. Following the second injection the patient began to clear up rapidly and at the present time is in complete possession of his senses, but has a hemiplegic residue. Eye grounds now normal. The spinal fluid in the second examination showed marked xanthochromia, cells reduced to 200 and a corresponding decrease in the other findings. The examination of the last cerebrospinal fluid showed 27 cells and the organic solids almost normal. The Wassermann, however, in the fluid is still positive.

Case 10. Tabes dorsalis, age 40. Syphilis eight years ago. Marked ataxia, lost knee and Achilles jerks, severe gastric crises, leading to extreme emaciation. Examination of the spinal fluid showed 78 cells and positive other findings throughout. Intravenous injection of salvarsan failed to control the crises.

February 4, 1915. Intradural injection one-third of a milligram of Old Salvarsan. Cessation of vomiting and nausea within six hours.

Patient writes back that he has gained 20 pounds in weight, has no lightning pains and is markedly improved in every way.

October 13, 1915. Patient returns. Wassermann on blood negative. Cerebrospinal fluid shows six cells entirely negative throughout. Patient has remained well and has had no crises and no pains.

Case 11. Cerebrospinal syphilis, gumma of the brain, age 20. Syphilis three months previously, malignant type followed by rupial eruption, general weakness in the legs and marked neuro-retinitis, marked increase in the tendon reflexes on the left side. Has had severe headaches and fits in which he falls to the street unconscious. Examination of the cerebrospinal fluid showed 40 cells and markedly positive other findings throughout. Blood Wassermann is also positive. Patient received one-third of a milligram of Old Salvarsan in the cerebrospinal canal on December 21, 1914. Two weeks later marked subjective improvement, neurological examination, however, unchanged. Examination of the cerebrospinal fluid showed 12 cells and correspondingly diminished other findings throughout.

Case 12. Tabes dorsalis, age 31 (Female). Syphilis eight years ago, Argyll-Robertson pupil, lost knee and Achilles jerks, incontinence of urine, shooting pains in the legs. Examination of the cerebrospinal fluid showed 160 cells and positive findings throughout. Blood Wassermann also positive.

June 28, 1915. Intradural injection one-third of a milligram of Old Salvarsan. Patient was discharged from the hospital two days later considerably improved in gait, but still some pain.

July 16, 1915. Patient returns much improved in health, she has some less pain. The spinal fluid shows 126 cells, Albumen +, Globulin +, Wassermann on the cerebrospinal fluid is plus-minus.

August 11, 1915. Patient returns markedly improved. She now no longer has any pains in the legs, the incontinence has been practically recovered and there has been a decided gain in weight. Examination of the spinal fluid shows 20 cells, Albumen negative, Globulin plus-minus, Wassermann still positive.

Case 13. Tabes dorsalis, (Female), age 31. Infection 13 years ago. Shooting pains in the leg, sluggish pupils, lost knee and Achilles jerk, slight Romberg, marked neuro-retinitis. Examination of the cerebrospinal fluid showed 23 cells, Nonne Apelt and Albumen show only a slight increase over normal. Wassermann on the spinal fluid positive.

July 1, 1915. Intradural injection of one-third of a milligram of Old Salvarsan was followed by no untoward symptoms.

July 12, 1915. Patient returned for further treatment and shows slight improvement. Intradural injection of one-third of a milligram of Old Salvarsan.

August 3, 1915. Intradural injection of one-third of a milligram of Old Salvarsan. Examination of the cerebrospinal fluid shows four cells, Albumen negative, Globulin negative. Wassermann on the spinal fluid ++. Following this injection the patient remained entirely free from all symptoms and she has gained decidedly in weight. The interesting feature in this case is the practical resumption to normal of the spinal fluid, paralleling the subjective improvement.

Case 14. Tabo-paresis, age 52. Denies syphilis. Pains in the legs of fifteen years' duration, shooting pains of the body, lost Achilles jerks, Argyll-Robertson pupil, marked ataxia, nervous irritability and emotional spells, neuro-retinitis. Examination of the cerebrospinal fluid showed 40 cells, markedly positive otherwise throughout. Patient received one intradural injection of one-third of a milligram of Old Salvarsan on August 16, 1915. This was followed a week later by a second puncture, showing eight cells, Nonne Apelt and Albumen both doubtful. Subjectively as well as objectively this patient was markedly improved. At the present writing the patient is subjectively much improved. He now has very little trouble in urination and is practically free from pain.

Improvement in this case should perhaps be discounted by the fact that the patient has paresis as well as tabes.

Case 15. Tabes dorsalis, age 39. Syphilis eighteen years ago. Marked ataxia, Argyll-Robertson pupil, impotence, marked tabetic pains, lost knee and Achilles jerks, marked hypotonia, unable to stand without assistance of two canes.

December 16, 1914. Patient received an intradural injection of one-third of a milligram of Old Salvarsan, followed by slight neuralgic pains. He returned to his professional duties and subsequently wrote there has been marked improvement in health, gets along with one cane, and there is complete cessation of lightning pains. Six months later the patient returned in person, walked over one mile to the office, still ataxic although markedly less so than before. Examination of the cerebrospinal fluid at first examination showed 60 cells,

markedly positive throughout. Six months later examination of the cerebro-spinal fluid showed 12 cells and markedly diminished other findings throughout. Wassermann on the blood and spinal fluid, however, is still positive. Patient received two injections with no untoward symptoms. Patient writes he continues to improve and is now potent.

CONCLUSIONS

1. Of the fifteen cases treated by this method none have suffered any ill consequences. In all but two cases very definite objective improvement could be noted in the spinal fluid.

2. With full allowance for the possible suggestive element there was nevertheless a very marked subjective improvement in the majority of the cases. Such improvement was not seen to be transitory but appears to be permanent.

3. Three cases have disappeared from observation, but none of the remaining twelve have relapsed with regard to symptoms. Four cases have been restored to usefulness and are making a livelihood, which before their treatment they were unable to do.

4. The cases showing the most marked improvement were those of early brain or cord syphilis, but encouraging results were also noted in cases of tabes dorsalis. No case received over three injections, but it seemed to the writer that further treatment in cases in which improvement had occurred would be indicated.

5. It would appear that if carried out carefully, the technique of this procedure is without danger to the patient. The method described above seems to possess decided advantages over that previously described by the writer.

6. The encouraging results detailed above are qualified only by a very short period of time that the patients have been under observation. In no case has more than a year elapsed and it was not without or beyond a possibility that relapses may take place. The writer is inclined to believe, however, that the acute cases at least have been permanently benefited.

The writer begs to acknowledge with thanks the cordial co-operation of his colleagues, Professors Canfield, Parker and Camp, in checking up the auditory, ophthalmological and neurological findings.

THE PHARMACOLOGY OF BRONCHIAL ASTHMA*

BY D. E. JACKSON, PH.D., ST. LOUIS, MO.

NOT only from the obscurity of its etiology, but also in the number and character of the remedies which have been used in its treatment, does spasmodic bronchial asthma present one of the most interesting chapters in modern medicine. Of the various theories which have been advanced regarding its cause, perhaps that which attributes its origin to a spasmodic contraction of the muscles of the bronchioles is now the most generally accepted. Its relation to other pathological conditions, such as hay-fever, spasm of the respiratory muscles, swelling of the bronchial mucosa, etc., we may pass over for the present, since the disease in its simplest form presents all the features which it is the object of this article to discuss.

Among the remedies which have been more or less extensively advocated for this condition may be mentioned morphine, chloral, cigarettes made from leaves of stramonium, belladonna or hyoscyamus, grindelia, cannabis indica, nitrites or nitrates, lobelia, tobacco, pyridine, scopolamine, atropine, hyoscyamine, chloralamide, inhalation of chloroform, diphtheria antitoxin, arsenic (arsenious acid, Fowler's solution, sodium cacodylate, atoxyl, etc.), ammonia fumes, eumydrin, caffeine, diuretin, hot mustard foot baths, steam baths, epinephrine, potassium iodide, various forms of hydrotherapy, electric light baths, Roentgen rays, compressed air, special breathing exercises, the selection of a climate suitable to each individual case, etc. Some writers claim to have seen good results follow the administration of pilocarpine.

It is impossible to state the manner in which many of these bodies might act to relieve the patient. With reference to a number of them, however, the action can easily be shown by pharmacological methods to be such as might readily benefit the patient. Similarly another possible feature of the disease which appears to have received practically no recognition in clinical literature may also be readily demonstrated pharmacologically. It would seem that most drugs which offer immediate help to the patient do one of two things. First, they may paralyze or depress the endings of the broncho-constrictor nerves in the muscle fibres of the bronchioles. This may cause the bronchioles to relax. The typical remedies of this group are atropine, scopolamine (hyoscyne), hyoscyamine, eumydrin, etc. Experimentally, the intravenous injection of one of these drugs may produce immediate and profound dilatation of the bronchioles which have been previously greatly contracted by the administration of certain other drugs (Fig. 1).

Nicotine and lobeline have been believed to cause broncho-dilatation by depression or paralysis (after a slight primary stimulation) of the ganglia lying in the course of the broncho-constrictor nerves. Experimentally, it can be shown that this action is so slight that it could not be expected to be of much

*From the Department of Pharmacology, Washington University Medical School, St. Louis, Mo.

use clinically. Second, drugs may relieve a bronchial spasm by a direct stimulation of the endings of the broncho-dilator nerves in the muscle fibres of the bronchioles. The typical drug producing this action is epinephrine. Others which have not been introduced into clinical medicine, but which act like epinephrine are -tetrahydronaphthylamine, trimethylamine hydrochloride, etc. The proprietary preparation "epinine" also has a similar action.

It is conceivable that some drug, such as amyl nitrite for example, might act directly on the bronchial muscle fibres and so depress them that a relaxation might occur in a manner analogous to the dilatation of the arterioles by the nitrites. But only slight emphasis has so far been laid on this possibility in practical therapeutics.

As intimated above, it can readily be shown experimentally that at least two separate and distinct forms of spasmodic bronchial asthma *may exist*. Whether or not these two forms do actually occur clinically does not appear to have received any attention by clinicians, but the experimental evidence when considered together with the unsatisfactory and variable results of treatment in various individual cases, would seem to make it extremely probable that at least two forms of the disease may exist separately, or that in some cases both forms may be present at the same time. Of these two forms one may be due to nervous influences, either central or peripheral and including reflexes. In this form, nervous impulses arising either from direct central influences or else reflexly excited by some form of peripheral excitation, such as diseases of the olfactory sinuses, etc., pass down the broncho-constrictor nerves and finally thus indirectly stimulate the muscle fibres to contraction. Or, chemical substances (e. g., injected drugs) circulating in the blood may stimulate these nerve endings (either directly or else indirectly by stimulation of the ganglia on the course of the constrictor fibres). Regarding the nature of any such chemical substances, which might be produced in the body and possess the power of stimulating the broncho-constrictor nerve endings, thereby producing bronchial spasm, we at present know nothing. Fig. 1 shows the production of this nervous form of broncho-constriction as the result of the intravenous injection of *arecoline*. Atropine completely removes this form by paralysis of the broncho-constrictor nerve endings. Neither arecoline nor any other drug producing the nervous form of constriction can have any further action on the bronchioles after a sufficient dose of atropine (see Fig. 2).

The other form of bronchial spasm is due to a direct stimulation of the muscle fibres of the bronchioles. It may readily be produced after enormous quantities of atropine or scopolamine. Consequently, if an asthmatic should be affected with this form of spasm, atropine might be of no benefit whatever so far as the bronchial spasm is concerned. Fig. 2 shows the action of dionine in causing a direct muscular broncho-constriction. Atropine injected during the contraction causes no dilatation. Injection of adrenaline, however, overcomes the constriction because adrenaline stimulates the broncho-dilator nerve endings (and the atropine has not affected these). A later injection of the same sized dose of arecoline as that which produced profound contraction in Fig. 1 now causes no contraction of the bronchioles whatever. This shows that after

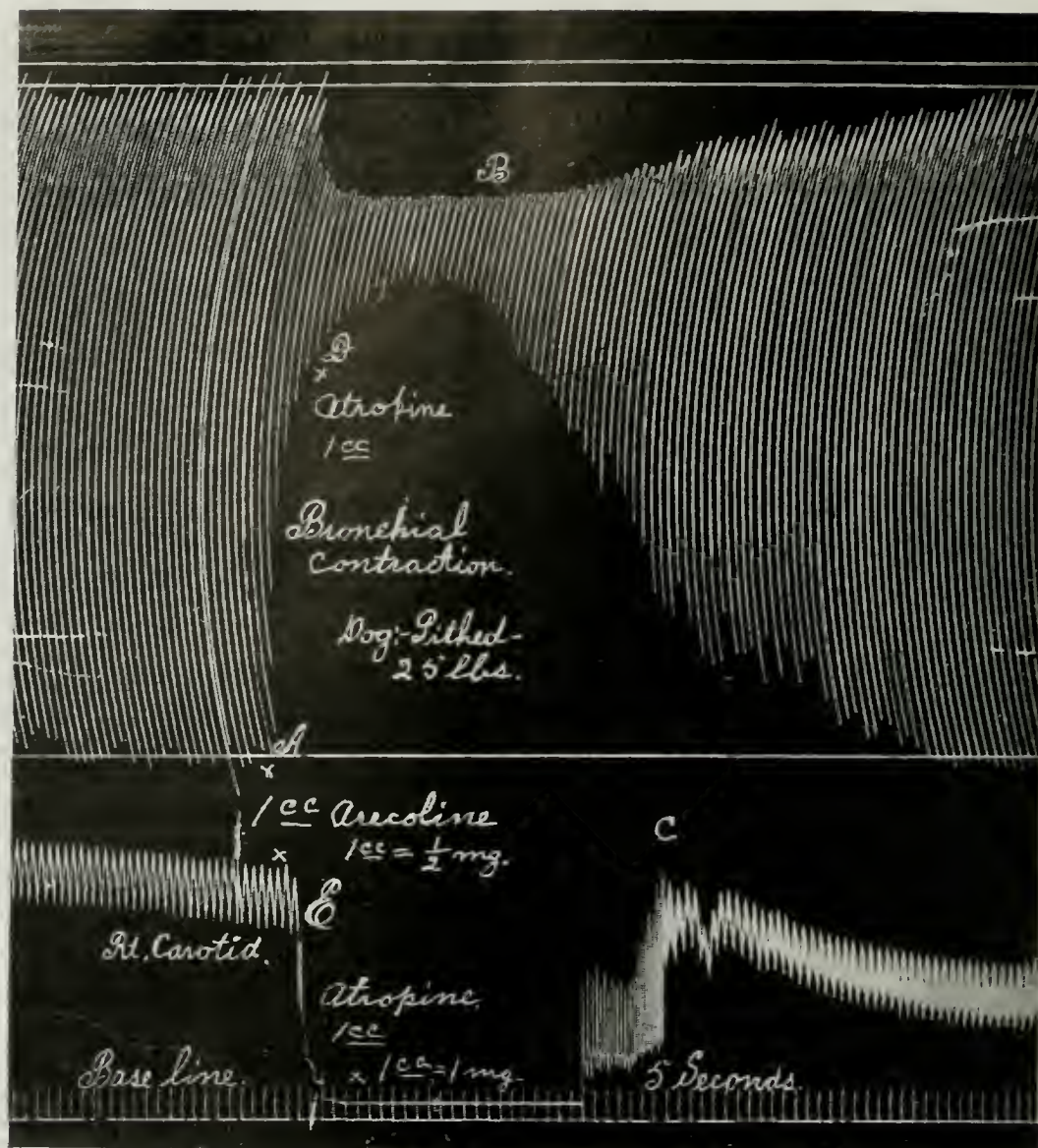


FIG. 1.

Figures one and two were both made in succession from the same dog. Under deep ether anæsthesia the animal's brain was destroyed by means of a probe passed through a trephine opening. The chest was then opened and a piece of apparatus* was inserted which held the chest walls rigid and in an expanded position. The chest was then closed airtight and air was intermittently aspirated out of the chest at about the rate of twenty-five times per minute. As the chest cavity was thus aspirated, air rushed into the lungs through the trachea thus producing inspiration. During the intervals between aspiration, air was allowed to enter the chest cavity through a tube in the apparatus. This entering air allowed the lungs to collapse of their own elasticity thus producing expiration.

From the side tube of the tracheal cannula rubber tubing led to a recording tambour which wrote on the revolving surface of a smoked drum. In this manner the upper tracing in each figure was made. The extent of the up and down strokes represents the amount of air passing out of or into the lungs respectively. The amount of this depends, under constant and regular force of aspiration, on the extent of contraction or dilatation of the bronchioles.

The lower tracing is the blood pressure recorded by a mercury manometer. In Fig. 1 one-half milligram of arecoline injected into the femoral vein caused marked bronchoconstriction by stimulating the endings of the broncho-constrictor nerves in the bronchial muscles (at A). This also stopped the heart by stimulating the vagus endings in the organ (E). One milligram of atropine (D) was then injected and soon paralyzed the endings of the broncho-constrictor nerves in the lungs and vagus endings in the heart. Thus the bronchioles were again able to dilate (beginning at B) and the heart again took on a rapid rhythm (at C).

*Jackson, D. E.: Journal of Pharmacology and Experimental Therapeutics, 1914, v, 479.

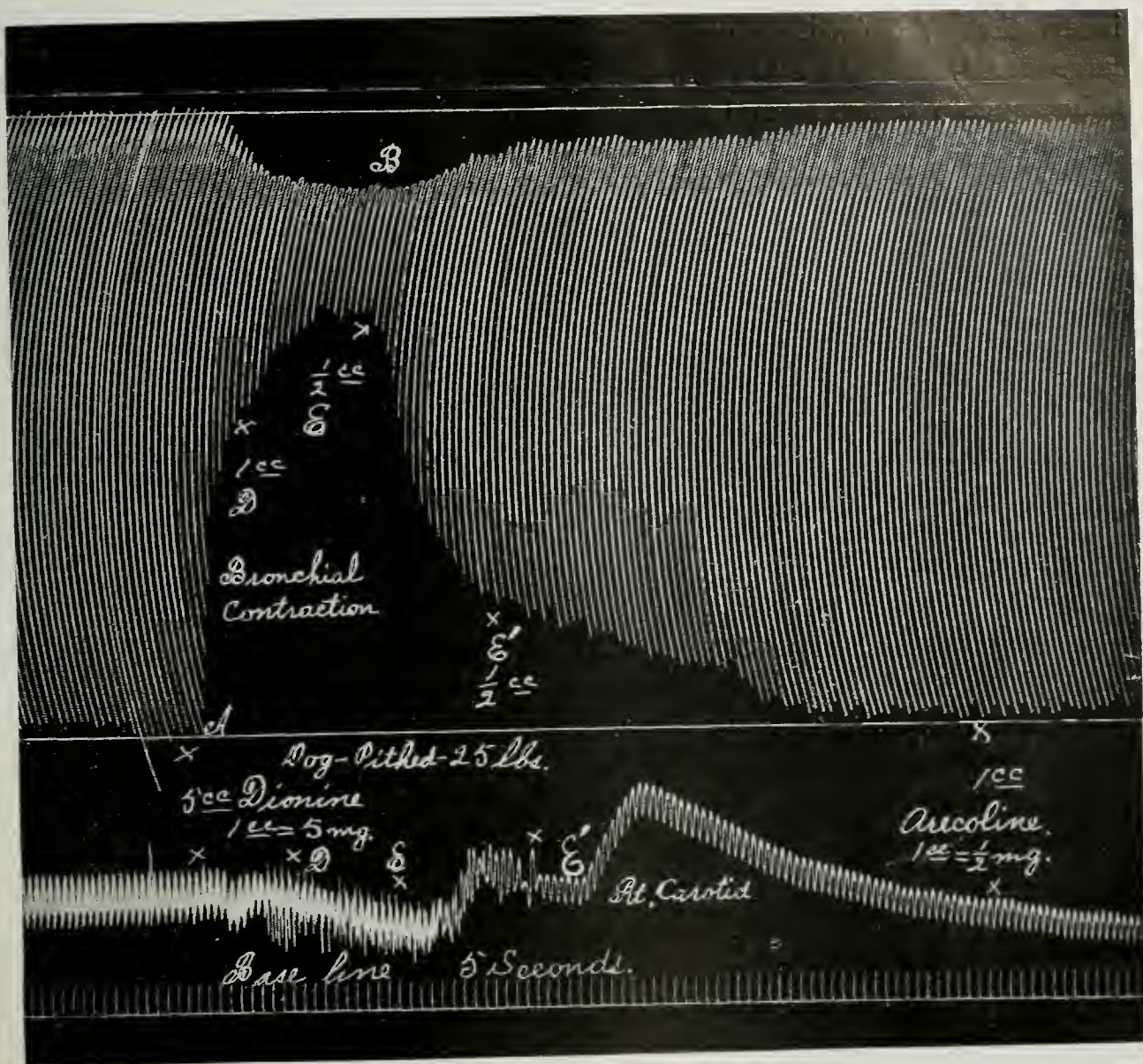


FIG. 2.

Directly after the broncho-constrictor nerve endings had been paralyzed (in Fig. 1), 5 cubic centimetres (25 milligrams) of dionine were injected. This at once produced a profound contraction of the bronchioles by a direct stimulation of their muscle fibres. The innervation of these structures had nothing whatever to do with this spasmodic contraction which is practically of the same order as that produced by arecoline.

Immediately following the dionine there was injected 1 cubic centimetre (at D, D) of atropine. This is the same sized dose of atropine (1 milligram) as that which in Fig. 1 caused dilatation after arecoline. The atropine in Fig. 2, however, had no effect at all on the broncho-constriction. Following this there was injected (at E, E) $\frac{1}{2}$ cubic centimetre of adrenaline. This was followed later by a further injection of adrenaline (at E', E'). These caused prompt broncho-dilatation by stimulation of the sympathetic broncho-dilator nerve endings in the bronchioles. Obviously in this form of bronchial spasm atropine would be a useless remedy. Later a further injection of one-half milligram of arecoline (the same as the original dose in Fig. 1) was given, but this produced no effect at all on either bronchioles or heart, thus showing that after atropine paralysis a nervous form of bronchial spasm is no longer possible.

a sufficient dose of atropine a nervous form of bronchial spasm is impossible, but the direct muscular form may readily occur. A considerable number of drugs are capable of producing this direct muscular spasm independently of the innervation. Among these may be mentioned most of the opium alkaloids¹ (in large doses) such as morphine, codeine, peronine, heroine, narcotine, etc., β -iminazolyethylamine, several metallic salts, etc. In many cases the direct muscular spasm produced by one of these bodies is just as abrupt and profound as that which could be caused by any substance acting on the nervous structures to cause contraction. But the spasm produced by muscularly acting substances is usually much more lasting in its nature and is as a rule more difficult to relieve by drugs with opposing action such as epinephrine which simply stimulates the broncho-dilator nerve endings.

It has been shown that guinea-pigs dying from anaphylactic shock are affected by profound broncho-constriction. Atropine has been found by Auer² to be of benefit in these cases, but is probably not quite so efficient as a full dose of adrenaline. It has been suggested that the substance produced in anaphylactic shock, and which in the guinea-pig causes broncho-constriction, may be β -iminazolyethylamine (histamine) or some nearly related compound. And it has been shown by Mellanby and Twort³ that certain bacteria which they were able to isolate from the alimentary canal, possessed the power of converting histidin into histamine (β -iminazolyethylamine). Experimentally, it has been shown that β -iminazolyethylamine acts directly on the bronchial muscle fibres (in dogs and cats), and that it is rather difficult for epinephrine to produce broncho-dilatation after large doses of the drug. Thus it would appear to be possible that β -iminazolyethylamine may be a source of broncho-constriction in man.

With reference to the use of pilocarpine, it is to be remembered that the intravenous injection of small doses in animals causes great broncho-constriction by stimulation of the peripheral nerve endings. On this basis it is difficult to understand how it could possibly act to relieve an attack of asthma, but Dale⁴ has recently shown that pilocarpine when injected into animals may stimulate the adrenal glands to an increased secretion. Possibly this may be the explanation of any (paradoxical) broncho-dilatation caused by pilocarpine. Cannon⁵ has also shown that nicotine may have a somewhat similar action on the adrenal glands by stimulation of the ganglia on the course of the sympathetic nerves supplying the adrenal glands. And Edmunds⁶ has shown that in other respects lobeline acts like nicotine. This offers a possible explanation for a slight relief from an asthmatic attack by means of lobelia.

In conclusion let us ask: Do there exist two separate and distinct forms of spasmodic bronchial asthma, the one of nervous, the other of muscular origin, and if so is it possible to differentiate between these clinically?

¹Jackson, D. E.: *Journal of Pharmacology and Experimental Therapeutics*, 1914, vi, 57.

²Auer, J.: *Journal of Experimental Medicine*, 1910, xii, 151.

³Mellanby, Edward, and Twort, F. W.: *Journal of Physiology*, 1912, xlv, 53.

⁴Dale, H. H., and Laidlaw, P. P.: *Journal of Physiology*, 1912, xlv, 1.

⁵Cannon, Aub and Binger: *Journal of Pharmacology and Experimental Therapeutics*, 1912, iii, 379.

⁶Edmunds, C. W.: *American Journal of Physiology*, 1904, xi, 79.

LABORATORY METHODS

TO Obtain Leucocytes.—Two general methods are employed in obtaining a large number of leucocytes for experimental purposes. One consists in throwing down the leucocytes from blood, kept fluid by citrate, in a centrifuge. This requires much blood and is a slow process. The other consists in injecting some foreign protein, such as peptone or bouillon or aleuronate in a body cavity. Dold (*Zentralblt f. Bakteriologie*, 76, 548, 1915) has shown that distilled water is quite as good. From three to six c.c. of sterile distilled water is injected into the pleural cavity of a rabbit. Twenty-four hours later the animal is bled to death, when the sterile exudate in the cavity will be found to contain as many as 140,000 leucocytes per cubic millimetre. Smaller amounts may be secured by injections of from 0.5 to 2 c.c. into the knee joints. The distilled water disrupts the cells with which it comes in contact and then serves as a foreign protein. The leucocytes thus obtained, under aseptic precautions, are sterile and may be used for phagocytic tests and demonstrations.

—V. C. V.

FOLIN'S Test for Sugar in Normal Urine.—Folin (*Jour. Biolog. Chem.*, xxii, 327, 1915) proposes the following demonstration of the presence of traces of sugar in normal urine. Two solutions are made: (A) Five grams of crystallized copper sulphate are dissolved in 100 c.c. of hot water and to the cooled solution are added 60-70 c.c. of pure glycerine. (B) One hundred and twenty-five grams of anhydrous potassium carbonate are dissolved in 400 c.c. of water.

To 10 c.c. of the urine add two grams each of picric acid and good quality bone-black (Kahlbaum's or Merck's blood charcoal), shake for five minutes and filter. The purpose of this procedure is to remove from the urine the creatinine which has some reducing power. The picrate of creatinine is formed and is held by the bone-black. In the absence of bone-black, picric acid alone may be added to the urine and the mixture allowed to stand over night. The picrate of creatinine will be deposited by this time and is removed by filtration.

One part of A and two parts of B are mixed in a test tube and to 10 c.c. of this mixture two c.c. of the creatinine-free urine are added. Two small pebbles are dropped into the tube to prevent bumping and contents are heated for one and one-half minutes with constant shaking.

"If the sugar present is considerable (above the normal variations) a typical reduction is obtained. If the trace of sugar is smaller, but still rather large, the whole solution will become turbid as in Benedict's test. If no such turbidity is produced and the boiling mixture remains clear, transfer at once (while still very hot) to a centrifuge tube and centrifuge for one to two minutes. Typical red cuprous oxide such as is obtained with pure sugar solutions will be found in the bottom of the centrifuge below the green crystalline potassium picrate which usually forms as the liquid cools."

A and B should be mixed only when ready for use and the mixture should be boiled. If on doing this a sediment is formed, the mixture should be boiled and filtered before the addition of the creatinine-free urine.

—V. C. V.

MARSHALL'S Method of Determining Urea with Urease.—As is well known, urea is converted into ammonium carbonate by urease. This ferment is a product not only of bacterial growth, but is found in some of the higher plants, being especially abundant in the soy bean (*Glycine hispida*). Marshall (Jour. Biolog. Chem., xiv, 283) first used urease from the soy bean in the quantitative estimation of urea.

The enzyme was first prepared as follows: Soy beans ground into a fine meal are kept in stock in wide mouth bottles. Twenty-five grams of this powder are mixed in 250 c.c. of water and allowed to stand with occasional agitation for one hour or longer; then 25 c.c. of $\frac{N}{10}$ hydrochloric acid are added and the mixture kept for a few minutes at about 35 degrees. The acid precipitates the mass of protein in the extract but has no effect on the enzyme. The precipitate is removed by filtration and the filtrate, containing the active enzyme, is put into a tube or bottle and treated with a few drops of toluene. This solution is alkaline to methyl orange and the degree of its alkalinity should be determined. Generally 2 c.c. require from 0.28 to 0.34 c.c. of $\frac{N}{10}$ hydrochloric acid for neutralization.

Van Slyke and Cullen (Ibid, xix, 211, 1914) have prepared powdered urease from soy beans and this has now become a commercial product. An aqueous extract of the beans is poured into ten times its volume of acetone when the ferment is precipitated, dried and ground into a powder. This retains its activity indefinitely.

Portions of urine of 1 c.c. are measured into test tubes, diluted to 10 c.c. with ammonia-free water, treated with the enzyme (1 c.c. of the extract from the bean or the same amount of a 10 per cent solution of the powdered enzyme). A drop of toluene is added to each tube. The well stoppered tubes are allowed to stand at room-temperature over night. The enzyme decomposes the urea into ammonium carbonate and the contents of the tube may be directly titrated with $\frac{N}{50}$ hydrochloric acid and the per cent of urea calculated; or the ammonia may be blown over into $\frac{N}{50}$ hydrochloric acid and the decrease in the acid determined.

Van Slyke and Cullen have slightly modified the method which they illustrate diagrammatically as follows:

- | | | |
|--|---|--|
| 1. Measure into A..... | { | 0.5 c.c. urine.
5 c.c. 0.6% KH_2PO_4 .
1 c.c. 10% urease.
2 drops caprylic alcohol. |
| Stopper A and allow to stand 15 minutes. | | |
| 2. Measure into B..... | { | 25 c.c. $\frac{N}{50}$ acid.
1 drop 1% sodium alizarin
sulphonate indicator.
1 drop caprylic alcohol. |

3. After standing fifteen minutes, aerate one-half minute. Then open A and add four to five GK_2CO_3 .
4. Aerate all NH_3 from A into B.
5. Titrate excess of acid in B with $\frac{N}{50}$ NaOH .
6. Calculate: $0.056 \times \text{c.c. } \frac{N}{50} \text{ acid} = \text{grams of urea} + \text{ammonia nitrogen per 100 c.c. of urine.}$

The ammonia nitrogen in the urine is determined in a companion tube which is not fermented. "The acid neutralized is multiplied in this case by the factor 0.0056, to give the per cent of ammonia nitrogen." This method is suitable for urines which contain not more than 3 per cent of urea, about the maximum in human urine. In working with the urines of dogs and cats dilution is necessary.

The urease method is especially suited for the determination of urea in the blood, since the ferment acts on no other constituent of this fluid. The blood as drawn, is mixed with one per cent its weight of solid potassium citrate to prevent clotting. If the determination is made on fresh blood, the amount of free ammonia is so small that it may be considered negligible.

"Three c.c. of blood are mixed with 3 c.c. of the 0.6 per cent KH_2PO_4 solution and 1 c.c. of the ten per cent urease solution. Five drops of caprylic alcohol are added and ten minutes are allowed for the enzyme to act. One measures 15 c.c. of $\frac{N}{100}$ acid into tube B. From this on the technique is the same as in the urine analysis except that the titration is performed with $\frac{N}{100}$ NaOH instead of $\frac{N}{50}$. The calculation is very simple because each c.c. of $\frac{N}{100}$ acid neutralized corresponds to 0.01 per cent urea."

"Grams urea per 100 c.c. blood $= 0.01 \times \text{c.c. } \frac{N}{100} \text{ acid.}$

Urea nitrogen per 100 c.c. blood $= 0.00466 \times \text{c.c. } \frac{N}{100} \text{ acid.}"$

The caprylic alcohol is used to prevent foaming. Amyl or ethyl alcohol or kerosene may be substituted but are not so good.

A clinical method consists in determining the increased alkalinity of the urine after fermentation, with methyl orange as an indicator. The direct method is described by Marshall as follows: Two 5 c.c. portions of the urine are measured into flasks of 200-300 c.c. capacity and diluted with distilled water to about 100-125 c.c. Two c.c. of enzyme solution (or 1 c.c. of a ten per cent solution of urease) are added to one flask, a few drops of toluene to each and the solutions allowed to remain, well stoppered, at room-temperature over night. The fluid in each flask is titrated to a distinct pink color with $\frac{N}{10}$ hydrochloric acid, using methyl orange as an indicator. The amount of hydrochloric acid required for the flask containing the urine and enzyme solution less the amount used for 5 c.c. of urine alone, and that previously determined for the 2 c.c. of enzyme solution (when the pure ferment is used, this correction is, of course, not needed) corresponds to the urea originally present in the sample of urine. Since 1 c.c. of $\frac{N}{10}$ hydrochloric acid is equivalent to three mg. of urea, the number of c.c. required multiplied by 0.6 gives the value of urea expressed in grams per litre of urine."

—I. C. I.

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Editor-in-Chief: VICTOR C. VAUGHAN, M.D.

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EDITORIALS

The Fight Against Tuberculosis in Michigan

THE legislature of 1915 placed \$100,000 at the disposal of the State Board of Health, to be used in the restriction of tuberculosis in Michigan. The State Medical Society inaugurated the work in a new way. The Governor at the request of a committee from the State Medical Society issued a proclamation stating that on August 20th each member of the Medical Society would examine anyone who presented himself without charge. Blanks were supplied the physicians and 438 examinations were reported. This figure does not represent the total number. Many physicians understood that they were to report only the positive cases. Moreover, many people who hesitated to accept a free examination went to the physicians on other days. This movement on the part of the physicians to serve the public has been fully appreciated.

The State Board of Health has selected a competent medical man to take charge of the campaign. He will have the direction of as many visiting nurses as may be needed. The county will be taken as the unit of the survey. Visiting nurses will go into each county and with the help of the local physicians will try to locate every suspected case. County clinics will be held in which these suspected persons will be thoroughly examined. All the physicians in the county will be invited to attend these clinics and to bring patients or persons suspected of having tuberculosis. After the survey in a county has been made, the ascertained facts will be laid before the supervisors and an attempt will be

made to induce each county to provide a hospital for the care of advanced cases and a full time health commissioner who with the assistance of visiting nurses will continue the work. The death rate from tuberculosis in Michigan is already lower than in any other state in the Union except Utah, and it is hoped that this campaign will lead to a still further reduction. This is a great work and it is to be hoped that the medical profession throughout the country will show itself to be big and wise enough to lead in the restriction and the final extermination of this preventable disease. There is opportunity for the profession to render the country a patriotic service which has come to no other class of men at any time or any place. To show ourselves practical leaders in the restriction of tuberculosis and other preventable diseases means the respect and gratitude of all thinking men and there need be no fear that the profession will not be amply rewarded for such service.

—V. C. V.

The Action of Smallpox Vaccine

TO the man who has not been vaccinated and has not had smallpox, the virus of this disease is pathogenic. To the man who has been vaccinated or has recovered from an attack of the disease, this virus is non-pathogenic. How does vaccination secure protection? In *variola vera* the infection is general and is distributed through all parts of the body in the blood. Animals have been successfully inoculated with the blood, bone marrow, lungs, testicles, and other tissues of the smallpox victim. In this way the disease has been induced in apes and locally in the cornea of rabbits. While the virus is generally distributed in *variola*, the predilection site for it is in the epithelial tissue where it forms pustules.

In *variola*, pustulation is multiple; in *vaccinia*, it is single. In the former the contents of a pustule, even when diluted a thousand times, induce *variola* when inoculated into a susceptible individual. In the latter the contents of the single pustule confer immunity when inoculated into a susceptible individual.

Is the vaccine virus distributed through the body in the circulation from the site of inoculation? This is an important question from either a theoretical or a practical view. Only in rare instances does multiple pustulation follow vaccination. So long ago as 1829 it was held by Eichhorn that the vaccine virus is distributed through the body in the circulation. He scarified the skin of children at points distant from the site of inoculation on the fifth day after vaccination with a "clean" lancet and saw vaccination pustules form at the points of scarification. A "clean" lancet in 1829 did not imply a sterile one. Strange to say, this statement of Eichhorn has never been satisfactorily confirmed or denied. It was confirmed by Wolffberg in 1885, but his statements leave one in doubt concerning his aseptic precautions and the possibility of infecting the scarifications from the primary vaccination by external agents rather than through the circulation. Eichhorn's experiment has found denial in those made with all needed precautions by Nohl, but for some reason, not stated, Nohl made the scarifications between the tenth and twentieth days after vaccination

and not on the fifth day as Eichhorn did. The belief that the vaccine virus is distributed through the blood and may cause pustulation in distant parts of the body wherever the skin is broken, has led to caution in vaccination of children with eczema. Undoubtedly such children in some instances develop multiple pustulation over the eczematous areas. It is most probable that these areas are infected through the fingers rather than through the blood. However, the evidence that the vaccine virus does find its way into the blood after ordinary vaccination cannot be ignored or lightly cast aside. In 1872, Richter applied a vesicator to an unvaccinated child, removed the skin from the blister and applied to the raw surface lint moistened with the blood of a child which had been vaccinated eight times, twenty-four hours before. At the same time he made five small incisions in the skin of this unvaccinated child and rubbed into these dried blood from the vaccinated child. On the eighth day after this procedure he saw eight well-developed cow-pocks on the blister and one in the incisions. Neisser reports successful vaccination with the bone-marrow of an ape taken fifteen days after the animal had been vaccinated. Pfeiffer, Frosch, Wasielewski, and others have reported the presence of the vaccine virus in the liver, spleen, kidneys, lungs, and other organs of vaccinated calves. The expressed juice from these tissues induced typical corneal vaccinia in rabbits.

On the other hand, numerous investigators have failed to find any evidence that the vaccine virus is distributed in the blood. Halterstädter and Prowazek used 228 animals with no proof of the distribution of the vaccine virus through the blood. Similar negative evidence has resulted from the experiments of Calmette and Guérin, Prowazek, Haaland, Hansen, Jürgens, Süpfle, and others. Some of these investigators have approached the subject from another direction. For instance, Prowazek and Yamamoto injected vaccine virus directly into the circulation and found that it disappeared from the blood within an hour and from the liver, spleen, and bone-marrow within two hours. It can be detected in the skin any time within two days after intravenous injections, as has been demonstrated by the experiments of Calmette and Guérin. The intravenous injection of vaccine virus in experimental animals is followed by no pustulation unless the skin be broken. Calmette and Guérin found that pustulation could be induced any time within twenty-four hours after intravenous injection in rabbits by shaving the back and rubbing the skin with sandpaper.

It is safe to conclude from the evidence that vaccine protein has its predilection for epithelial tissue, that it speedily disappears from the blood when thrown directly into the current and that its distribution through the blood is unusual or does not occur after ordinary vaccination.

That the vaccine virus does not enter the blood in vaccination in man is indicated by the fact that eczematous children may be vaccinated without the development of pustules in eczematous areas when these are protected against external infection. When large amounts of vaccine virus are used, some of it may enter the blood from which it soon disappears and is deposited in the epithelial tissues where it induces pustules only when this tissue has suffered some injury. In vaccination as ordinarily practiced in man the virus grows only at and about the point of inoculation. Accepting this as true, how is the immunity

induced by vaccination to be explained? Tanaka, Freyer and Casagramli have concluded from their experiments that the blood of the vaccinated and of convalescents from smallpox contains specific precipitins, but Pirquet and Prowazek have shown that vaccine lymph itself deposits a flocculent precipitate on standing. Jobling and others have studied complement fixation in the blood of the vaccinated and that of convalescents from smallpox, but the methods employed have varied so greatly and the conclusions reached have been so dissimilar and even contradictory that nothing definite has been established.

Attempts to passively immunize children and calves with the blood and serum of the vaccinated and of smallpox convalescents have been made by many, first by Chaveau and since his time by many others. Earlier experiments in this direction were wholly negative. In later years much larger amounts of blood have been employed—even transfusion in calves—and partial immunity has been secured in this way.

It was first shown by Sternberg in 1892 that immune blood, whether the immunity be induced by vaccination or by an attack of smallpox contains a specific vaccinicide. Sternberg's demonstration was made in the following way: Equal quantities of the immune blood and the vaccine lymph are thoroughly shaken in a test tube. The mixture or the sediment from the same is without effect on susceptible individuals. This experiment has been repeated and the finding confirmed by many. The serum of susceptible individuals has no effect on vaccine virus, while that of the immunized promptly destroys it.

The vaccinicide action of the serum is evident about the eighth day after vaccination and increases for three to four weeks. In some instances it persists for years, but this is not the rule. Even after the presence of the vaccinicide in the serum cannot be demonstrated, the skin retains its immunity. In fact, the skin immunity appears a day or two before the presence of the vaccinicide in the blood can be demonstrated and may persist for years after the blood vaccinicide cannot be demonstrated. It seems justifiable to conclude that the vaccinicide is formed in the epithelial cells.

The vaccine virus grows only at and immediately about the point of inoculation. In doing this it elaborates enough vaccine protein to sensitize the epithelial cells of the whole body. The sensitizing agent is not the living virus but the protein of the dead virus. That this is true is proven by recent experiments of Süffle and others which show immunity may be induced by vaccine lymph sterilized but without the production of a pustule. Of course this method necessitates the introduction of relatively a very large amount of the lymph. It must equal that produced by the living virus in the formation of the pustule. The sensitizing consists in developing in the epithelial cells a new function, that of digesting and destroying vaccine protein. Vaccine protein and smallpox protein are so closely related that a ferment which destroys one has a like effect upon the other. When the individual protected by vaccination is exposed to smallpox or takes the smallpox protein into his body, it soon finds its way to its predilection tissues, which consist of the epithelial cells. The cells now exercise the function developed in them by vaccination and destroy the smallpox virus before it has time to multiply to a dangerous extent. There seems to be no missing link in the chain of facts connected with the development of immunity to smallpox by vaccination.

—I. C. I.

Recent Work on Blood-Pressure Measurements in Man

(Continued from page 68.)

THE DIASTOLIC PRESSURE

AS we have already pointed out, much more is to be learned concerning the efficiency and condition of the circulatory system by measuring the diastolic pressure than by measuring the systolic. A knowledge of both pressures is important, and both should always be measured, but, taken alone, the diastolic is the more important pressure to employ in clinical diagnosis. Although every authority, nowadays, insists on this fact, yet there are comparatively few physicians who make diastolic pulse gauging a routine, or indeed even an occasional, practice. The reason for this neglect is undoubtedly the unsatisfactory and uncertain nature of the methods which, until recently, have been at their disposal. But such is no longer the case, and we shall endeavor in this review to explain the newer, accurate and simple methods and to show how various investigators, particularly Professors Leonard Hill and MacWilliam and their co-workers, have succeeded in proving the reliability of them. In general, two methods may be employed, the *oscillatory* and *auscultatory*. One procedure is common to both, namely, to apply an armlet to an artery that is well surrounded by soft tissues, such as the brachial in the arm or the femoral in the thigh. The pressure in the armlet is then altered by means of a suitable pump and is registered on a manometer, which may be either a spring instrument (such as the Hill-Barnard or Faught) or a column of mercury (such as the Riva Rocci or Janeway). In the oscillatory method, the diastolic pressure is taken as that at which the oscillations of the manometer are greatest in amplitude; in the auscultatory, it is taken as that at which a sudden change in intensity and character occurs in the sound that may be heard with each pulse beat by listening through a stethoscope or phonendoscope placed over the artery below the armlet, for example, at the bend of the elbow when the brachial artery pressure is being measured.

The Oscillatory Method. A most exhaustive investigation of the reliability of this method has been published by MacWilliam and Melvin,¹ and their general conclusion is that "the Marey principle . . . that the maximum oscillation occurs when the external (armlet) pressure counterbalances the internal diastolic pressure . . . is erroneous." Instead of this being the case, they conclude that "maximum oscillation occurs at levels of external pressure above and often very considerably above the actual internal diastolic pressure, the excess varying between a few m.m. Hg. and 20 m.m. or more."

These criticisms of the principle of the method are rendered more serious by the further conclusion that there is often no reliable way for ascertaining at what pressure the maximum oscillation occurs. Several methods have been in vogue for determining this point. These methods have been: (1) To take the midpoint in the period of approximately maximal oscillation—found to give widely varying overestimates. (2) The Recklinghausen-Erlanger index or the last phase at which the oscillation is maximal—found to be valuable only when a characteristic change in oscillation magnitude is clearly recognizable, and even in this case the diastolic pressure is that obtaining just after the abrupt diminution has occurred. (3) The Sahli, Masing, etc., method, in which changes

(observed by palpation or by sphygmographs) in the artery peripheral to the armlet are used—found to be dubious and the interpretation unreliable. The same is true when subjective changes, such as the throb felt in the limb, are used.

These conclusions are based on evidence obtained by experiments with an artificial schema consisting of a wide glass tube (the compression tube), filled with Ringer's solution, representing the armlet, and having a fresh artery running along inside it from end to end. The artery is attached to tubing through which occurs a pulsatile flow of oxygenated Ringer's solution at varying pressures, which are indicated by valved manometers connected with the artery tubing just beyond the compression tube. The pressure in the latter is also measured by a manometer and it is caused to vary by a suitable compressor. Fresh arteries are used in these newer artificial circulation models, because, as MacWilliam has shown, living arteries when subjected to changes in pressure inside or outside, do not behave like dead arteries or rubber tubes. Arteries are tubes of very special structure, and their expansile and elastic properties depend on their being alive.

By comparing the behavior of the artery with the pulsating movements of a spring manometer which was connected with the compression chamber, under different degrees of pressure inside and outside the artery (as ascertained by the appropriate manometers) the following was observed:

"The maximal oscillation only occurs when the artery is flattened between the pulse beats to a very considerable extent." That is to say, it occurs at an outside pressure which is above the diastolic, for at this pressure the vessel should retain its circular shape. A higher external pressure causes diminished oscillation by producing excessive flattening. So also does lowered pressure because the vessel, being circular between the beats, only expands into a wider circle with each pulse wave. It was noted, however, that often "the change between the latter form of expansion and that compounded of such expansion and the opening up of a partially flattened vessel is sharply marked and . . . constitutes the real basis of the Recklinghausen-Erlanger index." According to this observation, therefore, the diastolic pressure, as measured by the Erlanger apparatus, is the level at which the markedly diminished excursions are first obtained.

The authors speak of the vessel as being in zero position (of normal circular form with zero pressure inside); half-flattened or completely flattened. They have found that the half-flattened position is the optimum one for a systolic expansion to start from, i. e., it takes fewer m.m. Hg. pressure to produce a maximal oscillation with the vessel in this position than in any other.

We shall return to these experiments immediately, when considering the auscultatory method, but meanwhile we must note that Flack, Hill and McQueen⁸ have pointed out certain fallacies in the schema of MacWilliam and Melvin, and therefore in the conclusions which these authors have drawn. Flack, Hill and McQueen consider the model too simple since it does not represent the influence which the vascular tissues, enclosed in the armlet, will have on the pulse wave. As indicating the unreliability of the simpler model, they point out that in it a compressing force, which was insufficient to obliterate the pulse, caused a great fall in the manometer which was placed distally to the

compression. This would mean that, in the living subject, when the compressing force which was just below the systolic was applied (in an armlet applied at the upper arm), a second armlet placed below the first one (at the forearm) would register a systolic pressure which was much lower than the actual. This, however, is not the clinical experience, for the second armlet under such conditions actually registers the true systolic pressure. Thus, in a case in which the systolic pressure is 115 m.m. Hg., if a pressure of 110 m.m. is applied at the brachial, and another armlet put below the first and the pressure raised within it, the pulse at the radial will not be obliterated until the pressure in this armlet reaches 115 m.m. Hg.

They point out other inconsistencies in the model, such as the fact that with moderate compression it does not show the increase in amplitude of the pulse below the compression, which we have seen to be the clinical experience (see p. 66).

To represent the actual conditions, Hill, etc., have constructed a more elaborate circulatory model⁸ consisting of two glass compression chambers connected with each other and to a compression bottle. In one is placed a piece of human carotid artery, in the other a schematic representation of the tissue vessels consisting of a very thin-walled wide rubber tube filled with broken rubber sponge and supported on the outside by muslin. These essential parts of the model were arranged in different relationships to each other in the numerous experiments that were performed with them, and the pressure changes and pulse oscillations were recorded by connecting manometers, at various parts of the schema. The pulsatile flow, etc., was provided for in the usual manner. In the present connection the most important experiments were those in which the resistance to outflow and the diastolic pressure increased *pari passu* with the compression. In these, the diastolic pressure and maximal pulse were in agreement.

Other well-known clinical facts which could be duplicated on the schema were as follows: (1) "The pulse may reappear on decompression at a lower pressure than that at which it disappears on compression; (2) the radial pulse is reinforced when the compressive force applied to the upper arm is below the diastolic pressure; (3) the diastolic pressure is raised towards the systolic in proportion as the peripheral resistance is increased by obstruction of the venous outflow.

We have already seen, in connection with systolic pressure, how the model could also be used to demonstrate the difference which is often noticed in the leg and arm systolic pressures (see p. 65).

To sum up this part of the work, MacWilliam and Melvin assert that the diastolic pressure in the artery does not always coincide with the maximal pulse observed in the armlet manometer, whereas Flack, Hill and McQueen find that they are in agreement. As far as one can judge, without actually repeating the experiments, the balance of evidence is in favor of the views of the latter group of workers. This does not detract from the importance of MacWilliam and Melvin's work, for as we shall see immediately, it has proved of immense value in proving the reliability of the auscultatory method.

The Auscultatory Method: Both schools of workers are in agreement that the diastolic pressure corresponds to the pressure registered in the armlet when,

on gradually decompressing this, a sudden diminution occurs in the sound heard in the artery below the armlet.^{5 7}

To secure trustworthy results the following conditions must, however, be observed in using the method:

(1) The armlet should entirely surround the limb and should be at least 12 c.m. in width.¹

(2) It must be applied to an artery which is surrounded by vascular tissues, such as in the upper arm and thigh, and cannot be used on an artery lying on bone.^{1 7}

(3) The phonendoscope or stethoscope should be laid without pressure just below the armlet; on raising the armlet pressure above the systolic and then slowly decompressing, clear sounds become audible, with each heart beat, gradually getting louder and often murmurish in quality. At a certain pressure the loud sounds suffer a sudden diminution in volume and tone. The pressure in the armlet at this moment is the diastolic.^{1 7} Dull sounds continue to be heard on further lowering the pressure, but *these have no diagnostic significance*. The method may also be applied with gradually rising pressure, the point of sudden increase in the sound being the diastolic index.

(4) The systolic index should also be measured by the auscultatory method (see p. 64), but not only because it is important to know it, but in order that it may be compared with the systolic index as gauged by the reappearance of the pulse as felt by the finger. The auscultatory index should be above the palpatory; if it is not so, the apparatus must be readjusted.^{1 4}

(5) The method is not applicable in cases of aortic regurgitation, on account of the continued presence of the well-known sound.

(6) Too prolonged application of the armlet, by causing congestion and stagnation of blood in the limb, may cause the auscultatory method to be unreliable.¹

Alterations in the arterial wall do not interfere with the accuracy of the method unless they are severe. Insufficiency of flow through the artery and conduction of the sound along bone are, however, possible sources of fallacy.⁷

The exact condition of the blood vessels at the diastolic index can be beautifully demonstrated in MacWilliam and Melvin's simple model,¹ for if an auscultatory instrument be applied to the tube leading from the artery just beyond the compression chamber, sounds are heard which correspond to those heard in the arm, etc.

By watching the artery under different conditions of external pressure while listening for the sounds, it was found, on gradually lowering the pressure from above the obliteration point, that the sound begins as soon as a certain amount of fluid is forced through the compressed area at each pulse (the systolic index). As the flattening becomes less, the sound becomes louder and a murmur develops. When these are at a maximum there is seen to be extensive flattening with very obvious vibrations of the arterial walls, which may often cause a sensation of thrill on palpation. The sound suddenly becomes less and changes in character just exactly when the external pressure no longer produces any flattening of the vessel between the pulses: evidently, therefore, this is the diastolic pressure. As the external pressure is still further lowered, the sound gets less and less and finally disappears.

It must be clearly understood that it is the systolic wave that produces the sound that is to serve as an index of diastolic pressure. To quote MacWilliam and Melvin,⁵ "Though the sound is actually elicited by the systolic wave, its occurrence and non-occurrence and its character when present are determined by the intra-arterial pressure existing during the diastolic phase and its relation to the external or armlet pressure. If the intra-arterial diastolic pressure stands in such a relation to the armlet pressure that some distortion of the arterial walls from the circular form—that is, some flattening—occurs during diastole, then the loud, clear sound is heard. On the other hand, when the relation is such that the intra-arterial diastolic pressure is not sufficiently below the level of the armlet pressure to ensure some flattening between the pulse beats, the sound, if audible at all, is dull in character. *The change (during the lowering of the armlet pressure) from the clear to the dull and weaker sound, occurring as soon as the external arterial pressures are approximately equal, constitutes the index of the diastolic pressure.*"

Lastly it should be mentioned that Flack, Hill and McQueen⁷ in confirming MacWilliam and Melvin's findings have shown that there are really two elements that enter into the production of the sounds, viz.: (1) the vibrations which are set up in the arterial wall by the onrush and outrush of blood which is synchronous with each systolic wave; (2) sudden changes in the tension of the arterial walls produced by each systolic wave. The former, or vibration sounds, depend on the degree of constriction of the artery under the armlet, but the latter, or "tension" sounds, are independent of this and occur, therefore, after the pressure has been lowered below the diastolic.

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—J. J. R. M.

Indigestion

THAT the term indigestion covers a multitude of evils is fairly well known. That the term is used rather indiscriminately, and usually has a purely symptomatic meaning is fairly well realized. It is further, a term which may include anything from "stomach trouble" to flatulence. One reason, perhaps,

that "indigestion" is so nondescript is because the methods for arriving at an exact diagnosis are so tedious in some cases, and perhaps ill understood. Perhaps the physiology of the gastro-intestinal tract is not sufficiently understood by many. It is therefore a valuable contribution of Vander Hoof¹ which deals with 1,000 cases of "indigestion." These cases were studied thoroughly, and use was made of all the clinical methods including blood examinations, X-rays, and urine and gastric chemical methods. No reference however was made to routine examination of the feces.

The results of the study are summarized as follows:

Appendicitis and Cholecystitis	35 per cent
Peptic ulcer	10 per cent
Neuroses	10 per cent
Cancer (stomach or intestines).....	5 per cent
Chronic gastritis, visceroptosis, peritoneal adhesions, entero- spasm, enterogenous toxemia	10 per cent
Affections of the kidneys, lungs, heart, eyes, blood and ductless glands, ears, central nervous system, female pelvic organs, migraine and infectious diseases	25 per cent
Miscellaneous diseases	5 per cent
	<hr/> 100 per cent

In other words, in 25 per cent of the cases the symptoms of "indigestion" were reflex in origin, and were not the result of gastro-intestinal disease. Vander Hoof remarks that the percentages attributed to peptic ulcer and to the neuroses are probably too high, because in the cases of ulcer many of the cases were treated medically, and because a question mark follows the diagnosis of neurosis in many cases. He believes that many of the cases in these groups may have been the subject of appendix or gall-bladder disease.

Many of the patients who received surgical treatment for appendix or gall-bladder diseases have been followed with the following results:

	Patients Operated.	Patients Reported.	Per cent Well.	Much Improved. Per cent	Not Improved. Per cent
Appendicitis	145	93	58	29	13
Cholecystitis	60	41	68	25	7
Total	<hr/> 205	<hr/> 134	<hr/> 61	<hr/> 28	<hr/> 11

If one takes the above figures in which there is no doubt of the gastro-intestinal origin of the symptoms, he finds that 45 per cent of the 1,000 cases are definitely of infectious origin. If one adds to that figure such other groups as peritoneal adhesions, chronic infectious diseases, dysentery, diseased tonsils, intestinal parasites, diverticulitis and tuberculosis, he realizes that at least 60 per cent of the cases, and probably more, are of infectious origin.

Recent work, and particularly that of Rosenow, has indicated that cholecystitis, appendicitis and gastric ulcer are results of infection from foci of infections frequently outside the digestive tract. It is, therefore, not to be wondered at that treatment which merely aims at correcting gall-bladder or appendix conditions, for instance, results in but 61 per cent of cures. In the other 39 per cent of cases the sources of infection may not have been removed. One might suggest that intensive study of the teeth and cranial sinuses and antra might add to the value of studies on indigestion. —P. G. W.

¹Johns Hopk. Hosp. Bull., 1915 (20) 151.

Alkali Salt Therapy in Anuria

IT has taken a long time for the methods of M. H. Fischer for producing dehydration of protoplasm to be used in treatment of appropriate cases. A reason for this is that there has been difficulty in deciding what cases are appropriate. There can be no doubt that in those clinical conditions which are due to tissue oedemas with no other complicating causes the hypertonic alkaline salt solutions are valuable. In toxic anurias for instance, which are the results of local cellular effects in the kidneys, no one can have any doubt of the value of alkali treatment. On the other hand, in those cases in which the symptoms are the result of a combination of cellular and vascular effects (as in renal arteriosclerosis), brilliant results are not to be expected, for if the vascular conditions are such that the organ is only barely well supplied with oxygen, and if then the cells swell under the influence of some toxic influence which does not permit the cells to use what oxygen comes to them, alkaline and salts added to the blood will not reach the cells in sufficient amounts or in time to produce the dehydrating action upon which a return to former conditions depends. Furthermore, when a toxic material produces an irreversible change (coagulation) or the proteins of the cells (of the kidney, for instance, in anuria) neither salt and alkali nor any other drug can help.

In this connection a report by Woodyatt¹ is a valuable one. This case was one which became anuric after receiving three doses of salvarsan in eight days, in 0.3, 0.3, and 0.4 gram amounts. Evidently the arsenic produced cellular asphyxia which expressed itself renally in the absence of urine. Asphyxia is the equivalent of acidosis, and acidosis leads to colloidal swelling. The patient was treated with Fischer's solution by bowel, and sodium bicarbonate lemonade by mouth. Some few hours later the anuria disappeared. The first day 600 c.c. of urine passed; the second day 900 c.c., and at the end of the week the daily average was 1,500 c.c. "An isolated experience of this sort is considered as suggestive that a suitable application of the same general therapeutic principles might prove of value in intoxications of the type described."

Salt-alkali treatment has also been advised in other conditions which are supposedly due to overproduction of acids in the cells of the tissues. For instance, Wherry² advised it in cholera, in which disease its value has been studied and systematized to a certain extent by Sellards.³ Woolley⁴ also has suggested the use of alkali-salt solutions in insolation which is reasonably explained as an acidosis resulting from rapid metabolism due to heat retention in the body.

Evidently it is becoming more and more apparent that many of the conditions which we have been wont to call uræmia, nephritis, etc., are merely oedemas, and that these oedemas are the results of water retention by the protoplasm of organs which is brought about by increased presence of acids, and which may be controlled (and which is normally controlled) by salts. If the oedema is a cerebral one, uræmia results; if it is renal, we speak of nephritis. These as a rule are the result of the presence of abnormal amounts of acids in the tissues, or perhaps, of decreased salts,—at any rate, of relative increased acid content. Alkali will help to neutralize the acid and salts will dehydrate the protoplasm.

—P. G. W.

¹Woodyatt: Jour. A. M. A., 1915 (64) 1811.

²Wherry: Forchheimer's Therapeutics of Int. Diseases, 1912, Vol. IV.

³Sellards: Amer. Jour. Trop. Dis., 1914 (2) 104.

⁴Woolley: N. Y. Med. Jour., June 12, 1914.

Mutation in Bacteria

AS Sleeswijk (Weichardt's *Ergebnisse*, 1914) points out, the founders of the science of bacteriology held widely divergent views concerning the mutability of bacteria. Nägeli wrote: "The same species in the course of generations may differ greatly both in form and in function. It may cause the souring of milk, the development of butyric acid in sauerkraut, the ripening of wine, the putrefaction of proteins, the decomposition of urea, the fermentation of carbohydrates, cause diphtheria, typhoid fever, recurrent fever, cholera, and malaria." This certainly would be designated now as wholly untenable.

At the other extreme were the views of Cohn who taught that identification and classification must be determined wholly by the form. Pasteur, who was a chemist before he became a bacteriologist, concerned himself but little with the morphology of the organisms which he studied, but depended upon their functions. Koch whose dictum has largely dominated bacteriological studies depended largely upon cultural characteristics as shown in growths on solid media. The slightest variation in these has been deemed sufficient to proclaim a new species or at least a variety. At first special stress was placed upon the morphology of the individual bacterial cells, but soon it was seen that pathogenic and saprophytic bacteria may not be distinguished by form, while undoubtedly pure cultures showed marked polymorphism. Then attention was diverted to the forms of colonies, but like variation was soon evident in these.

In attempting a study of mutation in bacteria we must distinguish between those variations which are inherited and continued from generation to generation and those which are only temporary and undoubtedly due to wholly external conditions. As an instance of the latter we may mention the frequently observed fact that of a number of bouillon flasks inoculated with the same diphtheria culture, some will yield a rich toxin while others produce only a little. In this case there is some difference in the contents of the flasks. The experimenter may not be able to determine what this difference is, but there can be no doubt of its existence, because bacteria taken from a flask in which the production of toxin has been slight and placed in another often elaborates a large amount. Even more marked variations may be due to temporary conditions and should not be regarded as mutations. For instance, Bordet and Gengou have shown that the bacillus of whooping cough grows on a specially prepared medium consisting of potato, blood and agar. It also grows fairly well on ordinary agar. The growths on these two media show marked differences. One growth is known as the "blood bacillus" and the other as the "agar bacillus" and the serum of an animal treated with the "agar strain" agglutinates this strain, but not the "blood bacillus," while the serum of an animal treated with the "blood bacillus" agglutinates both strains. This difference, however, is not inherited and the "agar bacillus" when grown on the medium containing blood becomes the "blood bacillus," and vice versa. In other words, the differences between the two strains are wholly determined by the medium on which the bacillus is grown and are not due to any permanent alteration in the bacterial cell itself.

The terms, variation and mutation, have been used loosely and interchangeably. In discussing the subject of mutation in bacteria, I will restrict this term

to those changes in form or function which persist through one or more generations after the cause of the alteration has ceased to operate. In other words, mutation, as I will use this term, means alteration in form or function which is inheritable or transmissible to succeeding generations. To state it differently still, it means the inheritance of acquired characters. Does this occur in bacteria and if so what is the mechanism of its development? These questions are of importance for the light that they may throw upon the inheritance of acquired characters in multicellular organisms and for our guidance in the study of bacteria themselves.

Eisenberg (Weichhardt's *Ergebnisse*, 1914) has collected and discussed the literature of this subject. I will utilize the wealth of material which he has brought together, but will develop my own conclusions. Weismann taught that since bacteria multiply by fission, each descendant being half of the parent, the new generation cannot differ from the preceding and that in unicellular organisms the inheritance of acquired characters is not possible. In short, there can be no acquired characters, since the descendants in each generation are equal parts of the parent. Eisenberg properly refuses to accept this dictum and points out that each individual in the new generation must construct half itself out of the medium in which it lives. There must be a chemical reaction between the newly-born bacillus and the constituents of the medium in which it develops its other half. In this reaction is there not the possibility of an alteration in the inherited half? We will suppose for instance that a given strain of the typhoid bacillus has never been grown in a medium containing a given carbohydrate, consequently it has never fed upon or decomposed that carbohydrate. Now, plant it in a medium containing this new constituent, it develops the capability of utilizing or decomposing this substance. We can conceive of its doing this only through the action of a ferment which it has not previously used. It is true that it may have possessed this ferment before it had opportunity to use it, but when we find that with each transplantation it acts more energetically upon this carbohydrate, we must admit that quantitatively at least its function in this direction has been developed. A given bacillus is destroyed by a highly dilute solution of some poison, such as arsenic, mercury or quinine, but by transferring it from still more dilute to more concentrated solutions it finally becomes immune to this poison and one hundred times the strength originally fatal to the bacillus is now without effect. Does this not mean the development of a new character? Now, if the bacillus be grown through several generations in media free from this poison, and then transferred to a medium containing many times the originally minimum fatal proportion of the poison, and if it develops, does it not show that the acquired character has been inherited?

Twort took a strain of the typhoid bacillus which had no action on lactose or dulcitol, and after two years of training it acquired the property of fermenting these substances and it retained this acquired function after it had been grown on agar and passed through guinea-pigs. During this time it remained agglutinable with typhoid serum. When grown on litmus-lactose plates ninety-six per cent of the colonies were red. Typhoid bacilli capable of fermenting lactose have been found in the stools of typhoid patients. It is probable that this function is acquired in the intestines of patients fed on milk. However, this

function is rare in typhoid bacilli and there is no reason for supposing that this organism under natural conditions contains a lactose. Penfold has adapted typhoid bacilli to growth on media containing dulcitol. The first growths develop traces of acid within from five to fifteen days. After a month's training abundant acid is developed within from one to four days, and after two months the amount of acid developed within from one to three days may be sufficient to kill the bacilli. When this function of fermenting dulcitol has been fully developed, the bacillus retains it even after twenty-five transplantations on media free from this substance. In like manner the typhoid bacillus has been trained to ferment arabinose and rhamnose. Saisawa found that a strain which had been trained to ferment rhamnose retained this function after sixty transplantations on agar free from this substance. Ordinarily the typhoid bacillus decomposes glycerine with the development of acid, but in a small milk epidemic Mandelbaum found a strain which decomposes glycerine with the production of alkali. This unusual property was retained throughout a year of observation with frequent transplantation. Otherwise, in cultural properties this strain corresponds with the orthodox bacillus and it agglutinates with typhoid serum. In the dung of the cows from which the milk came this strain was found associated with the typical typhoid bacillus, but in the patients the typical bacillus could not be found. In the course of two years, Haendel and Baerthlein increased the resistance of the typhoid bacillus to quinine twenty-eight fold. It is worthy of note that in increasing the resistance of bacteria to poisons the progress is not along a uniformly ascending line, but that there are plateaus with no increase followed by abrupt ascents, such as are observed in the training of the nerve cells of man. The plasticity of the paratyphus and Gärtner groups seems to be unusually great and many mutation types with changes in both form and function have been reported. I will refer to only one. Broddaert found that a typical paratyphus strain, shown to be homogeneous, decreased in agglutination to its homologous serum after passage through one rabbit, decreased more after passage through a second and failed wholly to agglutinate after passage through a third rabbit. This alteration was still in evidence after six months.

The chromogenic bacteria, such as the prodigiosus, are easily converted into colorless growths by high temperature, the presence of inhibiting substances and the exclusion of air. In some instances this variation persists through many generations after return to usual conditions. Whether such a loss of function even when inheritable, should be regarded as a mutation is a question about which there is marked difference of opinion among students of this subject. For my purpose it makes but little difference whether the alteration manifests itself in the acquisition of a new character or the loss of one hitherto possessed. In either case I hold there is a change in intramolecular structure.

Mutation has been observed so frequently in the colon bacillus that several strains have been reported under the name of *coli mutabile*. This is easily understood when we think of the widely variable and diverse conditions under which this bacillus develops in the intestines of different species of animals and in different individuals of the same species. In many gastro-intestinal disturbances the stools are found to contain colon bacilli which differ from those of normal stools in one or more characteristics. These departures from the usual may be manifested in form, in virulence, in fermentative activity, and in motil-

ity. These unusual characters often persist through many generations of growth on ordinary culture media. Such mutations have been observed by Massini, Burk, Muller, Penfold, and others.

The relation between diphtheria and pseudodiphtheria bacilli has not been conclusively shown. Fox (*Jour. Med. Research*, xxxii, 309, 1915) has reviewed this subject and concludes that there is no proof that a mutation has been secured artificially, but there is reason to believe that diphtheria bacilli in the animal body become atypical, lose in virulence, and vary in form and cultural properties. The diphtheria bacillus ferments glucose, maltose and dextrine but never saccharose; the pseudobacillus ferments saccharose, maltose and dextrine. The former varies widely in virulence and may be avirulent; the latter is only slightly virulent, though it may cause endocarditis, angina and otitis. Römer (*Ber. klin. Wochenschrift*, 1914, 503) starting with typical diphtheria strains, believes that he has secured transition forms by frequent passage through guinea-pigs. This supports the older findings of Bernhardt and Paneth. Many investigators agree that atypical forms are often present in the blood and urine of those ill with or convalescing from diphtheria. Trautmann and Gätgens took a typical pseudodiphtheria bacillus, which they found in the nasal secretion of a child sick with diphtheria and claim that they converted it into a typical diphtheria bacillus. This is important if true, because while the conversion of a diphtheria bacillus into a pseudoorganism may be regarded as a degenerative change, the reverse cannot be so considered.

Probably the most notable and striking demonstration of mutation in bacteria is seen in the work of Rosenow (*Journ. of Infectious Diseases*, xiv, 1, 1914), who has converted hemolytic streptococci from a wide range of sources, such as erysipelas, scarlet fever, puerperal fever, arthritis, tonsillitis, cow's milk, etc., into strp. viridans, strp. mucosus, and typical pseudopneumococci; strp. viridans into strp. mucosus, strp. hemolyticus and strp. rheumaticus; strp. mucosus into strp. viridans and strp. hemolyticus; strp. rheumaticus into strp. viridans and pneumococci. "In order to meet the objections that even though every ordinary precaution was taken to obtain pure cultures, I was working with mixtures whenever mutation was observed, cultures of each main variety were obtained from single organisms by the Barber method. The same results were obtained with three of these pure line cultures of hemolytic streptococci, six of strp. viridans, and two each of strp. mucosus and pneumococcus. Hence the changes observed are not due to mixtures or to so-called 'mass selection' but to actual changes wrought under the influence of changed environment. The transformation of some of the strains has been found to be complete by every test known. Thus, the morphology, the presence of capsule, the fermentation process, the solubility or insolubility in bile, and in salt solution, the behavior toward the respective broth culture filtration (Marmorek's test), the specific immunity response, as manifest by the production of opsonin and agglutination by anti-pneumococcus and antistreptococcus serum, and the more or less specific pathogenic powers have been studied. Strains that corresponded to hemolytic streptococci have been converted into typical pneumococci as determined by all the above tests and vice versa. The changes observed have frequently the characteristics of true mutations because they appear suddenly, under conditions more or less obscure and because the newly-acquired properties persist unless

the organisms are again placed under special conditions. A pre-mutational stage seems to be necessary because the same strain will not yield mutants when placed under what seem to be identical conditions at different times. The underlying conditions which tend most to call forth changes are, first favorable conditions for luxuriant growth and then under unfavorable conditions—under stress or strain. These seem to call forth new or latent energies which were previously not manifest and which now have gained the ascendancy and tend to persist. This fact makes it difficult to obtain mutations outside of the body with highly virulent strains, because they die before there is opportunity for the organisms to adjust themselves to the new conditions. It explains also why injection into cavities makes for greater changes than intravenous injections of moderately virulent organisms. Apparent mutations in animals have been observed almost exclusively in closed cavities such as joints and pericardium, and here mostly when the tissues of the host were gradually getting the upper hand and the organisms were being destroyed. The mutations in vitro may be spoken of as retrogressive and those observed in animals as progressive because in the former, virulence, fermentation powers and other evidences of a vigorous vegetative life are diminished, whereas in the latter they are usually increased."

Evidence of mutation in bacteria might be multiplied many times. It is shown in changes in form, in capsule formation, in the production of spores, in alteration in virulence, etc., but I think that I have collected enough data to conclusively show that in bacteria acquired characters are in part at least inheritable. The mechanism of this will be discussed in a subsequent number of this journal.

—V. C. V.

Etiology of Hodgkin's Disease

ONE of the difficult problems for bacteriologists in the last two years has been that of passing judgment on the etiological relationship between the diphtheroid organism, first cultivated by Negri and Mieremet in Europe, and very soon afterward in this country by Bunting and Yates, from Hodgkin's disease. Fraenkel and Much had described small rod-shaped granules or "splinters" morphologically found in the material obtained from Hodgkin's glands in 12 out of 13 cases after treatment with antiformin. These first observers believed their rods to be closely related to or possibly derivatives of tubercle bacilli. Negri and Mieremet,¹ in cultural studies of similar glands, obtained diphtheroid rods, on special media, under aerobic conditions, which suggested to them etiological relationship since, of course, for many years the infectious nature of Hodgkin's disease had been suspected. Bunting and Yates² in 1913 published a preliminary note in the *Archives of Internal Medicine* in which they announced the cultivation of a pleomorphic diphtheroid obtained on Dorset's egg medium and on glycerine phosphate agar. Later it grew on "ordinary agar" and could be cultivated aerobically or anaerobically. In some of their tubes there was associated with this organism a white staphylococcus. They mention another case obtained through Dr. E. C. Rosenow in which the cultivation was

¹Negri and Mieremet: *Centralblatt f. Bakt.*, orig. vol. 68, 1913, p. 202.

²Bunting and Yates: *Archives Internal Med.*, vol. 12, 1913, p. 236.

carried out on Loeffler's medium. Bunting and Yates³ immediately extended their observations by animal experiment, and reported in the same year that by repeated injections of these cultures into the right axilla of a monkey they were successful in producing progressive enlargement of the axillary lymph-nodes. The histology of the nodes was "characteristic of the early stages of Hodgkin's disease," as was the blood picture of the monkey. In the year following they report⁴ that the histological picture of glands taken three months after the inoculation of the monkey "leaves no question as to the relation of the lesion to that of human Hodgkin's." They also reported that they could artificially increase the virulence of the organism obtained by them.

Meanwhile the study of these organisms had also been taken up by Rosenow, and Billings and Rosenow⁵ in 1913 reported on their study of 12 cases in which they obtained organisms which corresponded with those of Bunting and Yates and that of Negri and Mieremet. In all but three cases other organisms were associated with the diphtheroids. Incidentally in this paper of Billings and Rosenow there is recorded an observation startling to the general bacteriologist in view of the authoritative position of the writers, since it records a very striking instance of bacterial mutation from the bacillus to the coccus form which tends to shake the long established belief in the stability of morphological type in the kingdom of bacteria. They state that single colonies on dextrose agar which showed bacilli only in smears yielded in sub-culture a pure culture of staphylococcus aerobically, and forms of the bacillus either pure or in mixture anaerobically on the same medium. "These facts," they say, "suggest strongly that the associated staphylococcus is derived from the bacillus." This in itself, if upheld, would constitute a most important bacteriological discovery. It would seem that further study on this point should be made either by the writers or others because of the entirely new possibility infused into bacteriological work and current opinions thereby. Vaccine treatment by these authors apparently yielded favorable results. In 6 cases there was uniform and rapid decrease of the size of the glands, "one of these," they state, "without Röntgen treatment." Billings and Rosenow do not feel absolutely convinced apparently since they felt unjustified at the time of writing in omitting Röntgen therapy in their cases. They suggest that if finally the microorganism isolated from the lymphnode is found to be the cause of Hodgkin's disease the use of specific vaccines without other treatment might become a proper form of treatment. Bunting and Yates in the following year feel more positive. They state their belief as follows: "Since our experiments demonstrate that the diphtheroid organism is pathogenic for the monkey, that it produces a progressive enlargement of the lymphnodes with lesions similar to those of Hodgkin's in man, and further that the blood changes in the monkey are similar to those in man, we feel fully assured of the etiological relationship of the diphtheroid organism (bacterium Hodgkini) to Hodgkin's disease." These results are, of course, of the most vital importance and if upheld would constitute a notable achievement in medicine.

The great importance of these communications has led to a considerable

³Bunting and Yates: Jour. Amer. Med. Assn., vol. 61, 1913, p. 1803.

⁴Bunting and Yates: Jour. Amer. Med. Assn., vol. 62, 1914, p. 516.

⁵Billings and Rosenow: Jour. Amer. Med. Assn., vol. 61, 1913, p. 2122.

amount of work within the last two years. Lanford⁶ isolated diphtheroids from Hodgkin's glands, from tuberculous glands, and from lymphosarcoma. Steele⁷ in 1914 obtained such organisms from a case of lymphatic leukemia. Rosenow⁸ himself in the same year in a study on the isolation of bacteria from tissues, reported the finding of diphtheroid-like organisms from the blood in the febrile period of four cases of Hodgkin's disease, but also the finding of similar organisms from the blood as from the nodes in two or three cases of erythema nodosum. He also obtained diphtheroid organisms from the blood in 4 cases of infections resembling rheumatism. He adds here that "a diphtheroid bacillus showing a wide range of preference for oxygen in the different cases has been cultivated in from some 40 odd cases of Hodgkin's disease." Summarizing his work, Rosenow expresses himself strongly in favor of etiological relationship between the diphtheroid organisms and Hodgkin's disease, but adds the saving clause that "the fact that other organisms are commonly found also and that diphtheroid bacilli indistinguishable from some of the strains from Hodgkin's disease are found in many other conditions compels one to wait for more experimental evidence before concluding that these bacilli are the real cause of Hodgkin's disease." Of great interest, therefore, are the recent studies of Rhea and Falconer,⁹ of Bloomfield,¹⁰ and of Fox.¹¹ Rhea and Falconer obtained a diphtheroid organism from Hodgkin's, but up to the time of writing obtained no results in monkey inoculation. Bloomfield made a careful bacteriological study of the contents of normal and diseased glands. He studied glands from a number of different conditions including Hodgkin's disease, sarcoma, and arthritis, and found that although bacteria were more common in the diseased ones he could also obtain bacteria from 2 out of 7 normal glands. He obtained a type of small gram-positive bacillus roughly corresponding to the diphtheroid described by others, in 4 cases of carcinoma glands, in 2 of Hodgkin's, in 2 of lymphosarcoma and in 2 of arthritis. In 4 other cases he obtained cocci. Since some of the organisms he isolated corresponded to the saprophytes normally found on the surface of the body, he concludes that it is not impossible that the lymphatic glands may occasionally filter out such microorganisms, or that possibly there may even be a saprophytic flora which is commonly found in lymph glands without being associated with disease. His facts as well as those of Rosenow's study would seem to indicate rather more definitely that we may be dealing with some such condition as that described by Adami as sub-infection, i. e., the presence of microorganisms in the circulation and tissues without causing any acute illness, than that we attribute definite etiological relationship with any disease to organisms cultivated from diseased lymphnodes.

Fox has compared many diphtheroid organisms, obtained by himself and sent to him by others, by extensive morphological and cultural experiments. He sums up by saying that "diphtheroid rods may be isolated from Hodgkin's disease and other adenopathies, but there is no uniformity in biology and morphology among strains isolated by three observers from clinical and pathological

⁶Lanford: *Am. Jour. Tropical Dis. and Preventive Med.*, vol. 2, 1914, p. 191.

⁷Steele: *Boston Med. and Surg. Journal*, vol. 170, 1914, p. 123.

⁸Rosenow: *Jour. Amer. Med. Assn.*, vol. 63, 1914, p. 903.

⁹Rhea and Falconer: *Archives Internal Med.*, vol. 15, 1915, p. 438.

¹⁰Bloomfield: *Archives Internal Med.*, vol. 16 (No. 2), 1915, p. 197.

¹¹Fox: *Archives Internal Med.*, vol. 16, 1915, p. 463.

Hodgkin's disease." Diphtheroid rods similar in biology and morphology to these are found in Hodgkin's disease, in cases of chronic atrophic arthritis and other conditions. He concludes that "more facts are demanded to show the exact relation of the diphtheroids to Hodgkin's disease." He obtained no favorable results with vaccination; Bloomfield's experience in this respect was that autogenous vaccination seemed to have no effect whatever, either beneficial or harmful.

Reviewing critically then the present status of the bacterial etiology of Hodgkin's disease, we do not feel that acceptance of the diphtheroid organisms as the etiological factor is as yet justified. In fact, were it not for the definite conviction based on histological study especially of such an experienced pathologist as Bunting, we would summarize the bacteriological evidence as rendering such etiological relationship unlikely. —H. Z.

Transmission of Pneumonia

IT may be remembered by older physicians that some of the European clinicians of the preceding generation, notably Johanessen, on purely clinical evidence advocated the isolation of pneumonia in consideration of the possibility that the disease may be transmitted from one person to another. The frequency with which pneumococci have been found in the mouths of normal individuals has until very recently seemed to preclude such a possibility, it seeming more likely that the infection might proceed from the patient's own mouth flora under conditions of depression of resistance or other localized vascular changes that favored the penetration of the pneumococcus into the lung. The studies of the last few years which have shown that pneumococci, though superficially alike, may nevertheless be separated, by agglutination reactions and protection experiments on mice, into a number of different types have made possible a return to the earlier point of view. It has been pointed out by these studies that the first three groups are disease-producing and give rise to three-quarters of all cases of lobar pneumonia. A fourth group, which is responsible for about one-quarter of pneumonia, are not easily distinguished from those normally found in human mouths. The other three, however, are easy to distinguish from the relatively non-virulent ones habitually inhabiting the mouth and pharynx. The highly virulent forms, moreover, of the first three groups, seem to grow in normal mouths only under special conditions. Dochez and Avery¹ have found in pneumococcus infections with type I and type II the same microorganisms may appear in the mouths of members of the family or nurses in close attendance upon the cases. Such attendants may be "carriers" of this type for periods as long as 39 days, the average, however, being less than this. Patients recovering from pneumonia may harbor the organisms for considerable periods, varying in these studies from 12 to 90 days counted from the onset of the pneumonia. It is pointed out that both the healthy carrier who has obtained his organism from the case and the patient himself after recovery, may be active carriers of virulent pneumococci. Inasmuch as these pneumococ-

¹Dochez and Avery: *Journal of Exper. Med.*, vol. 22 (No. 1), 1915, p. 105.

ci are distinctly different from those found in the mouth normally, it is not at all unlikely that pneumonia may, like typhoid, diphtheria, and some other infections, be transmitted from person to person, at least insofar as the passing of the specifically virulent pneumococcus strain is concerned, this giving rise to the actual disease only in cases in which such transmission is coincident with physiological depression favoring the development of the infection.

—H. Z.

Angina Pectoris

SINCE the time when Jenner bet that a certain case of angina pectoris would show coronary sclerosis at autopsy,—and won, his belief that the disease was an expression of arteriosclerosis affecting the coronary arteries of the heart has been shared by very many physicians. It is believed that under such conditions of vascular sclerosis, the heart muscles receive barely enough blood for its customary needs but is immediately embarrassed when extraordinary demands are made upon it, and it becomes locally anemic, and probably dilated, and pain results, exactly as it does in intermittent claudication, with which condition angina pectoris was compared by Potani in 1870. There are, however, too many instances in which no coronary sclerosis could be demonstrated to say that it is the sole cause of the complex. It has been found associated with acute dilatation of the heart, in which condition it is said that pain results from strain put upon the visceral nerves of that organ. It has also been found in association with neuritis of the cardiac nerves; with vaso-motor conditions which have produced transient cardiac conditions analogous to those caused by coronary sclerosis, and with neuralgias.

Hirschfelder¹ gives the following tabulation of the conditions most frequently encountered in association with angina pectoris:

- I. Organic lesions.
 - A. Coronary sclerosis.
 - B. Aneurism, especially of the first part of the ascending aorta, and of the sinuses of Valsalva.
 - C. Valvular lesions, especially aortic insufficiency.
 - D. Adherent pericardium.
- II. Vasomotor anginas.
 - A. Hysterical.
 - B. Toxic, especially tobacco, and caffeine.
 - C. Those associated with hyperthyroidism and exophthalmic goitre.
- III. Anginoid pains occur in cases of acute cardiac dilatation of healthy heart, as a result of primary cardiac overstrain.

If one considers only the anatomical lesions which are associated with cardiac angina, one sees at once that they may be grouped as of fundamentally arteriosclerotic and as dilatations.

Vaquez, in a recent article,² discusses the subject of angina pectoris and comes to the conclusion that there are two main types of the disease, one arterial which results from aortitis, and one cardiac which results from dilatation. He then goes further and states his belief that both are symptoms caused by dilata-

¹Hirschfelder: *Diseases of the Heart and Aorta*, 1913, p. 374.

²Vaquez: *Archives des Maladies du Cœur*, 1915 (8) 45.

tion; the one of the aorta, the other of the heart. He calls attention to the fallacy of the statistical method upon which the coronary theory is partially based, and upon the anatomical data which have been used to substantiate it, for he says not only is the percentage of cases of true angina associated with coronary sclerosis too few, but that a large percentage of cases of coronary sclerosis, which have no pain, and also there are not a few cases of typical angina in which the coronaries are normal. Furthermore it is known from experimental work that obstruction of the coronary circulation leads to profound modifications of cardiac rhythm, and these do not occur in the active type of angina pectoris. He therefore concludes that when coronary sclerosis occurs in angina pectoris, it is merely an epiphenomenon which indicates the location and type of arterial conditions which is more probably causal in its relation to the disease. He calls attention to the fact that nowhere are the filaments of the cardiac plexus as abundant as about the origins of the aorta and of the coronaries. In diseased conditions of the aorta, such as fibrosis and lack of elasticity, any strain which tends to dilate the aorta stimulates these nerves and results in pain,—in “the angina which is the cry of suffering of the sick aorta.” To account for the pain of dilatation, Vaquez says that the sudden dilatation of the heart places a strain, which acts as an irritant upon the myocardial branches of the cardiac plexus. He disposes of MacKenzie’s theory of cardiac exhaustion by calling attention to the fact that while there is a grain of truth in it (because perhaps cardiac dilatation is a sequel of myocardial fatigue) there is also a fallacy, based upon the observation in cases of angina of arrhythmia with alternating pulse, and a sudden great fall of blood pressure, both of which are well known symptoms of myocardial weakness. The difficulty is that while both may occur in angina by ventricular dilatation, in which they invariably follow the pain, never precede it, they are as a rule absent in the aortitic form.

There is much of value in this communication of Vaquez, but one great value is that it tends to simplify a subject which has shown a tendency to become too complicated.

—P. G. IV.

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ORIGINAL ARTICLES

GUMMA OF THE PITUITARY ASSOCIATED WITH LESIONS IN THE SPINAL CORD WHICH MAY REPRESENT EARLY LESIONS OF SYRINGOMYELIA*

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

IN the case which I shall report, two interesting lesions were found; one, a gumma of the pituitary, the other, a condition which possibly represents an early stage of syringomyelia. It is reported first because of the infrequency of both lesions, and second because of the interest that attaches, always, to early stages of processes which lead to clinical complexes. I regret the brevity of the history. It is possible that, under other circumstances, a more complete one could have been obtained, and it is possible also that some symptoms might have been discovered which could have been of interest in connection with the lesions.

The patient was one R. B., a colored male, aged 52. He was admitted to the Cincinnati General Hospital (No. 190,172) on December 7, 1914, when he offered the complaint of pain in the stomach and sore throat. The family history was not taken. In childhood he had had measles and mumps; in his adult years he had contracted typhoid, pneumonia, influenza, gonorrhea and syphilis. He had been an excessive smoker, but denied alcohol and other drugs.

The illness which resulted in his application for admission to the hospital began, he said, about three weeks before admission (November 15, 1914), at which time, he said, he had pain in the stomach accompanied by vomiting. At that time he had a sore area on his right leg about the ankle. He also had a "lesion" on the soft palate. He "did not speak plain." The occipital region of the scalp was normal. While in the ward he had a good appetite.

That is the extent of the history. There was no note of any symptoms nor of any treatment. No clinical diagnosis was made.

The patient died eight days after admission (December 15, 1914), and the post mortem was made eighteen hours after death. The weather was cold, and the body remained in a good state of preservation.

*From the Mary M. Emery Laboratory of Pathology of the University of Cincinnati and the Pathologic Institute of the Cincinnati General Hospital.

The following is the autopsy protocol:

The body was that of a colored man of apparently about 55 years of age. It weighed about 160 pounds and was in a good condition of nutrition. There were some old, irregular, partially healed, sluggish, ulcerated patches on the right shin near the ankle. The scalp was the seat of a generalized alopecia, so that it was almost completely bald. The skin of the areas of alopecia was white, pearly or opalescent in appearance and diffusely thickened. The pupils were equal, and neither dilated nor contracted. There was no edema of the legs. The peripheral lymph glands were not noticeably enlarged, though shotty to the touch.

When the scalp was stripped from the calvarium, an abundant serous fluid ran from the vascular foramina of the bones, and when the skull-cap was removed it was found that the dura and the membranes beneath it were filled with an extraordinary amount of a clear serous fluid. The dura was congested, the pia was not, but the latter was so saturated with fluid that, after removal of the dura, it stood up in blebs. There were no unusual adhesions between the membranes. The venous sinuses were not distended with blood. The convolutions were rather flat. The whole brain was pale, and showed no evident increase in consistency. It was placed at once in ten per cent formalin. Later it was examined and showed nothing macroscopically wrong, except that the pia was more than normally adherent. The falx was almost completely ossified from about the middle of the median fissure to the crista. The ventricles were not dilated. The chorioid plexuses appeared healthy. The sinuses were patent but about them the dura was thickened.

The same condition, which was noticed in the membranes of the brain, existed also in those of the cord, i. e., a tremendous edema. The cord was fixed in formalin and later was examined. It then appeared that the meninges were more than usually adherent and that in the pia here and there were spicules and platelets of bony material. There were no distinct macroscopic lesions in the cord.

The pituitary gland was removed and during this operation, which was difficult because of adhesions about it, it ruptured and disclosed a thick, yellowish, almost caseous material, which seemed to involve almost all of the organ, which was finally removed together with part of the sellar bone.

When the thorax was opened, the lungs did not collapse because of the presence of old bilateral adhesions. On the left side these adhesions were mostly apical and posterolateral. On the right, they were general. On both sides the lobes were adherent by old adhesions. There was no fluid in the pleural cavity. The thyroid was apparently normal. The thymus was atrophic. The bronchial glands were enlarged, succulent, deeply pigmented and edematous. The mediastinal tissues generally were edematous. Section of the lungs showed no apparent abnormality save a more or less generalized fibrosis, localized chiefly under the pleura.

The heart and thoracic aorta were removed *en masse*. The heart itself was atrophic and beneath the epicardium was a considerable increase in fat. The cavities were filled with a clotted, red, friable mass; the valves were apparently normal. The aorta was the seat of a well marked syphilitic aortitis in all stages of progress, from the early succulent pearly condition to a very distinct atheroma with calcification in plaques. In the ascending aorta, about

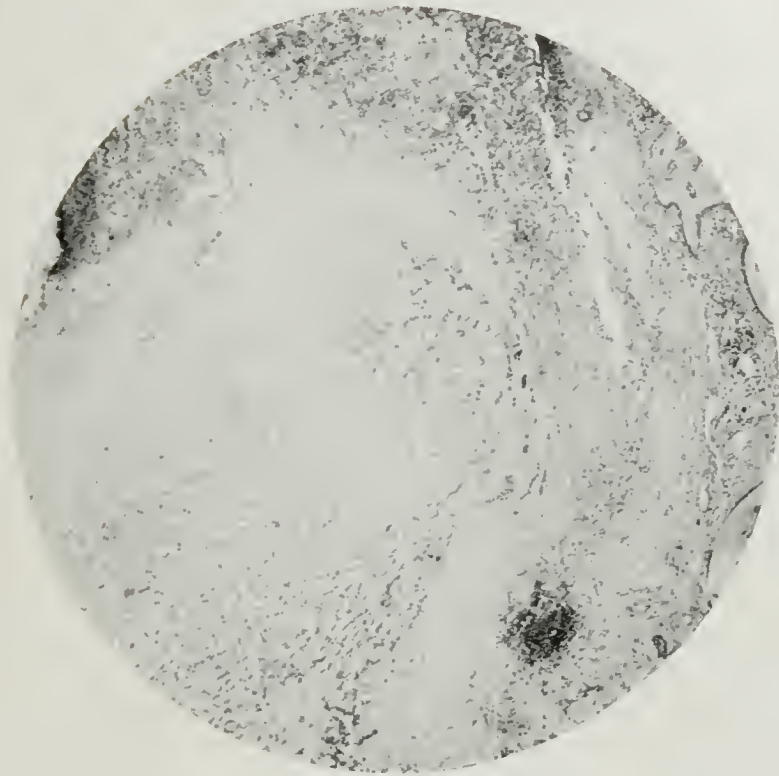


Fig. 1.—A portion of the gummatous lesion in the pituitary, showing also a remnant of pituitary gland, and a small miliary gumma. (By Dr. Charles Goosmann.)



Fig. 2.—A section of the cervical cord showing an area of necrosis which has apparently occurred in a luetic focus. (By Dr. Charles Goosmann.)

half way between the valves and the arch, was a saccular aneurysm, incomplete in form, which projected posteriorly and mesially, pressing upon the pulmonary artery which was bulged inwardly. This sac was about the size of half a hen's egg. Just beneath it was a smaller and more incomplete projection representing also an imperfect aneurysm. Upon the edges of the larger sac and also upon the edges of occasional atheromatous areas were masses of fine recent vegetations.

As one passed down the aorta, the atheromatous process became less well marked until in the abdominal aorta it was almost absent,—there being represented merely by some fatty degeneration in the intima. The process was, then, limited chiefly to the arch of the aorta and to the ascending portion. The sinuses of Valsalva were not involved to any considerable extent. The celiac axis and its branches were involved in the arteriosclerotic process and the renals also were involved to a less extent. The myocardium was pale and rather thin, particularly at the apex. The coronary vessels were slightly sclerotic.

The kidneys were small—each weighed 100 grams. They were pale, the capsules were adherent, the surfaces were roughened. The cortices were thin; the line of demarcation between the cortex and medulla was distinct; the glomeruli could be seen as small, glistening, translucent points and were not congested. There was a slight increase in renal pelvic fat. The ureters were apparently normal. The suprarenal bodies were cavitated. The bladder and prostate were apparently normal. The abdominal organs lay in their normal positions, and, except for the fact that the sigmoid was somewhat redundant, there was no obvious abnormality.

The appendix was long, patent and ran directly across the peritoneal cavity, reaching about an inch to the left of the spinal column. There was no obvious abnormality discovered in the stomach nor in the intestines.

The pancreas was apparently normal.

The spleen weighed 75 grams. It was atrophic, but aside from a moderate sclerosis, showed no obvious abnormality. The peritoneal lymph glands were not obviously enlarged. They were firm and congested.

The liver (900 grams) was atrophic and soft, though not increased in friability, and had a generally reddish color. Upon the surface were several stellate scars, the larger one occupying the central part of the right lobe. At the tip of this scar, in the substance of the liver, was a small caseous area forming the apex of the scar. Another stellate scar appeared at about the middle of the right lower edge of the liver which was deformed so that a notch was formed at this point. At the apex of this scar, also in the substance of the liver, was a yellowish, apparently caseous, area. Upon cutting the liver, the surface showed nothing more than a general reddening,—a moderate congestion,—with some slight increase in fibrous tissue. Along the portal vessels was a decided increase in fibrous tissue which had a rather pearly appearance, almost cartilaginous. The gall bladder contained a thick mucoid, pale, yellowish-colored bile.

Anatomic Diagnosis.—Edema of the brain and the meninges; Chronic diffuse nephritis with secondary contraction (arteriosclerotic); Aortic atheromatosis with aneurysm formation; Generalized arteriosclerosis; Syphilis (gummatous) of the liver; Syphilitic lesion (gumma) in the pituitary.

Microscopic Diagnosis.—Gummatous (luetical) hypophysitis; and hepatitis;

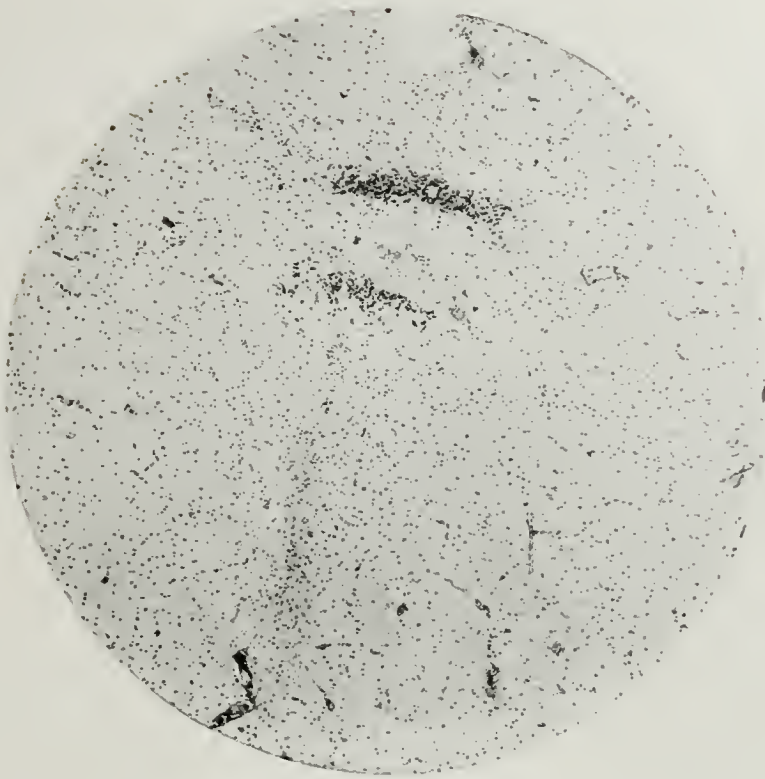


Fig. 3.—A section of the dorsal cord showing a collection of cells posterior to the central canal and its group of hyperplastic cells. (By Dr. Charles Goosmann.)

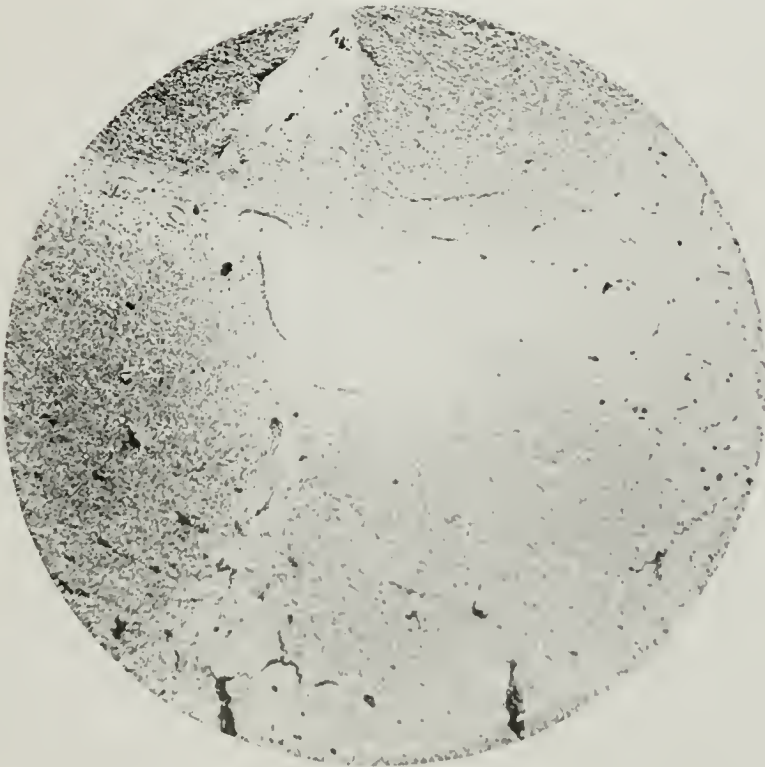


Fig. 4.—Section of the dorsal cord showing dilatation of the central cord. (By Dr. Charles Goosmann.)

Chronic diffuse nephritis, renal arteriosclerosis; Syphilitic dermatitis; Pancreatic fibrosis; Congestion of the spleen; kidney, liver, and pancreas. Degenerative (luetie?) lesions in the spinal cord.

In the following remarks on the histologic changes, attention is focussed upon the pituitary and the spinal cord, for there seems to be no obvious value in detailing the changes in all the other organs. Concerning these, it will suffice to say that the hepatic lesions were typically syphilitic, and that the aortic ones were typical of a luetic mesaortitis.

The sections showed that except for a small area of glandular tissue, the pituitary was almost replaced by a mass of gummatous tissue which was in large part necrotic (Fig. 1). Beyond this main lesion there were other smaller, more recent, younger, gummata, lying at the periphery of the larger lesion and beyond it in connection with the periosteum of the sellar bone which was the seat of a syphilitic periostitis. In none of these sections were tubercle bacilli found.

The following are notes on the cord lesions:

Sections made from the cord 32 c.m. from the tip of the cauda.—In these there is a general dilatation of the blood vessels of the anterior median fissure. The substance of the anterior commissure is edematous and in it there is a decided increase of small round cells of glial origin. The region of the central canal is represented by a mass of hyperplastic cells which, from their appearance, have originated in canal epithelium, and the central canal is represented by a mere slit. Posterior to this mass of cells are dilated blood vessels, posterior to which is an area lying to one side of the line of the posterior fissure which is apparently both edematous and necrotic. This area extends laterally and posteriorly, but chiefly laterally, and almost parallel to the line of the commissure. At its median extremity, which is broader than at any other point, a blood vessel appears, and about it there are a considerable number of round cells. In this neighborhood there is less necrosis and more edema, but laterally the round cells become scant in numbers, and the necrosis increases, until almost as the posterior horn is reached, the number of cells increases and necrosis decreases. Whether or not the above description is successful, the picture is one which suggests a linear focus of hyperplasia of glia tissue with central necrosis, and in some respects has a certain resemblance to what one might expect of a luetic focus. The tissue on the opposite side of the posterior fissure presents no such picture, and except for evidence of mild edema, appears normal (Fig. 2).

Sections made 19 c.m. above the tip of the cauda.—In these sections the commissure is generally edematous and more evidently so than in those taken higher in the cord. In this section the blood vessels are not only dilated but are surrounded by a clear zone in which there is considerable coagulated protein. The central canal appears as a small space surrounded by a complete epithelium, but laterally, for some distance, there are masses of hyperplastic cells springing evidently from the cells of the central canal. There is a second collection of these cells posterior to the first and separated from it by a very edematous tissue containing dilated blood vessels (Fig. 3). Extending from a point midway between and lateral to these two masses of hyperplastic cells is an area of degeneration within which there are corpora amylacea.

Sections made 18 c.m. above the tip of the cauda.—In these the central

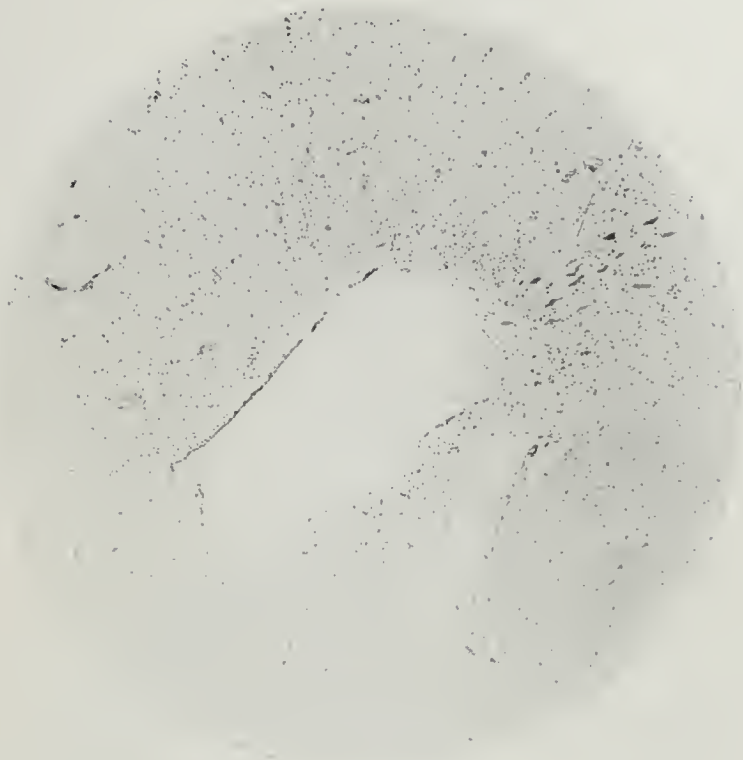


Fig. 5.—Section of the dorsal cord showing dilatation of the central cord. (By Dr. Charles Goosmann.)



Fig. 6.—A section of the dorsal cord showing a linear or somewhat fan-shaped area lateral to the central canal which is bounded by hyperplastic glial cells, and which shows a central area of degeneration. (By Dr. Charles Goosmann.)

canal is considerably dilated (Figs. 4 and 5) and lined with a cuboidal, or even a flat cuboidal epithelium. About it are a moderately increased number of small round cells.

Sections made 17 c.m. above the tip of the cauda.—In these sections there is again hyperplasia of central canal cells extending laterally from a small central canal, about which there is very distinct edema, associated with vascular dilatation. Extending postero-laterally from region of the central canal is a linear area almost destitute of cells in its center but surrounded by small round glial cells which are arranged in irregular columns which rest upon the degenerated area and extend at right angles to it (Figs. 6 and 7).

Sections taken from 16 c.m. above tip of cauda.—In these sections there appear to be two central canals, one in the usual position, and one lateral to it.

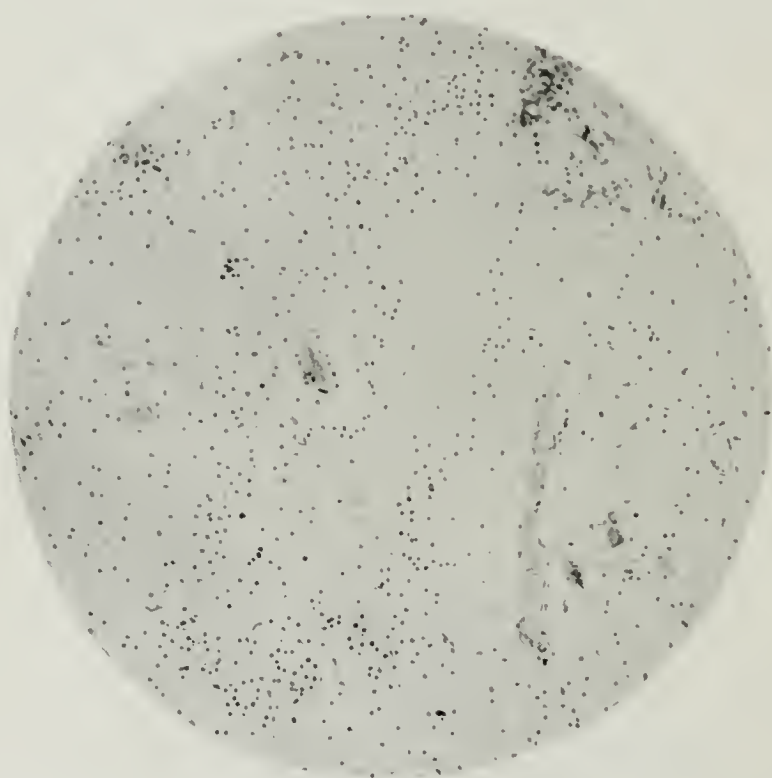


Fig. 7.—Same as Fig. 6, but more highly magnified. Shows the area referred to under Fig. 6. (By Dr. Charles Goosmann.)

The one is large, dilated and lined with low columnar and cuboidal epithelium; the other is small and lined with apparently normal epithelium. Between the two there are a few epithelial cells. There is, in these sections, no evidence of necrosis.

Sections taken 10 c.m. above the cauda.—In these, there is no evidence of a central canal but at its usual location is a collection of cells of central canal origin. Posterior to this cell mass and running posteriorly and laterally, is a linear area of gliosis which looks much like a scar. In it there is evidence of degeneration of a slight degree (Fig. 7).

DISCUSSION.

The meaning of these changes is not entirely clear, but looked at in one way, they suggest that in this case, syphilis, in addition to the lesions of the parenchymatous organs and of the pituitary, has also affected the spinal cord so that here and there hyperplasias of the cells of the central canal have been

initiated and have given rise by pressure to scattered dilatations of the central canal, with the production of a mild grade of hydromyelia. They also suggest that the same luetic process has been limited at certain foci which appear as linear areas of gliosis, with or without central necrosis.

If one wishes to assign the spinal lesions to one of the categories into which all cases of well-developed syringomyelia are grouped, one must choose that in which the primary syringo-myelias are placed. This case does not (provided it is actually an early stage of syringomyelia) belong in the group of neuro-epithelial glioses; it is not the result of diverticula of the central canal; there is

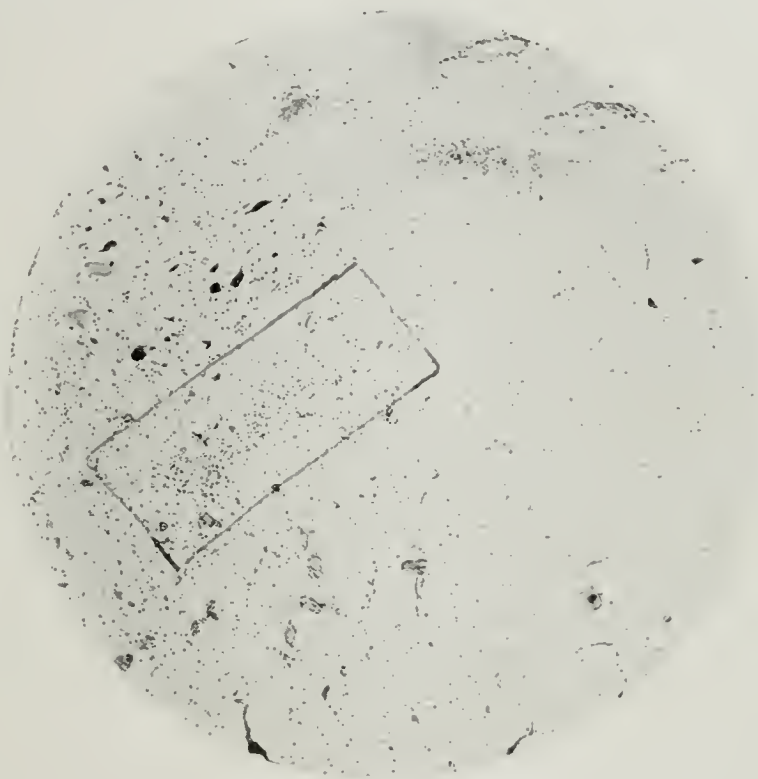


Fig. 8.—A section of the upper lumbar cord in which there was obliteration of the central canal and an area of gliosis postero-lateral to the region of the central canal. (By Dr. Charles Goosmann.)

no evidence of an anomaly of closure of the posterior sulcus, and there is no evidence that the lesions are the result of degeneration in gliomas.

CONCLUSION.

1. A case is reported in which there are certain lesions in the pituitary and in the spinal cord which, it is suggested, have a common etiology.

2. The lesion in the pituitary is definitely luetic. Those in the cord are possibly luetic.

3. The cord lesions seem to suggest that they represent an early stage of syringomyelia.

LITERATURE.

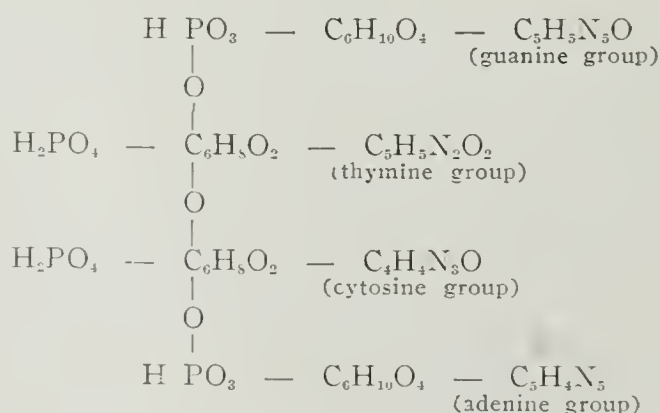
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SOME FEATURES OF PURINE METABOLISM*

BY H. GIDEON WELLS, M.D., CHICAGO, ILL.

THERE is much justification for the statement with which Walter Jones prefaces his splendid review of the nucleic acids,¹ "The nucleic acids constitute what is possibly the best understood field of Physiological Chemistry." The success with which chemical investigators have disentangled the many complex problems presented by the nucleoproteins is indeed remarkable, in view of the great difficulty of the task, and it is no small source of satisfaction to be able to record that much of the recent progress, built upon the fundamental studies of Miescher, Kossel and Emil Fischer, has been made in American laboratories, especially those of Walter Jones and P. A. Levene. We now have a definite understanding of the structure of nucleic acid, and we are able to state with some assurance the steps by which it is disintegrated in the animal body, a knowledge that has been given to us within a relatively short time. It is my purpose to present mainly certain aspects of purine metabolism that have been under investigation in my own laboratory for some years, although, to make clear some of the points involved, I shall need to preface the discussion of our work by a brief recapitulation of the newer facts concerning the chemistry and metabolic career of nucleic acid.

A long series of careful analytical studies has at last shown us that nucleic acids are, whatever the source, quite similar in composition, consisting always of a complex containing phosphoric acid, the two amino purines (adenine and guanine), two pyrimidines (either cytosine and uracil or cytosine and thymine); and a carbohydrate, which may be either a pentose or a hexose. Apparently there are two sorts of nucleic acids, one from plants, which contains always uracil and pentose, and one from animal tissues, containing instead thymine and a hexose. So constant are the findings in regard to these compounds that it has seemed feasible to consider their manner of union in the intact nucleic acid molecule, and Levene and Jacobs have proposed as the structure of thymus nucleic acid the following arrangement:



It will be seen that this proposed formula postulates in the nucleic acid

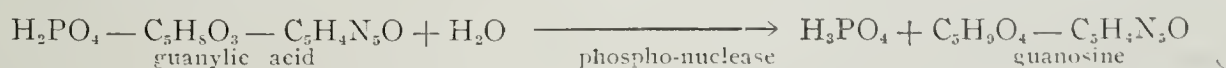
*From the Department of Pathology, University of Chicago, and the Otho S. A. Sprague Memorial Institute. Presented to the Chicago Society of Internal Medicine, Oct. 25, 1915.

¹Nucleic Acids, their Chemical Properties and Physiological Conduct. Monographs on Biochemistry, Longmans, Greene & Company, 1914.

molecule, one radical of each of the two purines and pyrimidines, each of these being united by a carbohydrate radical to a phosphoric acid radical. Recognizing that this must be looked upon as a provisional formula, it will serve as a base of departure from which to consider the metabolism of nucleic acid.

The grouping of hexose + purine or hexose + pyrimidine is referred to as a "nucleoside," analogous in terminology to "glucoside." The same groupings plus the phosphoric acid radical constitute the "nucleotids," nucleic acid thus being made up of four nucleotids. Within the past year Emil Fischer has reported² the synthetic production of a nucleotid, composed of phosphoric acid united to a glucoside of theophyllin, this really constituting the long-sought synthesis of a nucleic acid, even though the artificial product is not the same as any known to occur in nature. With these facts before us we may consider the manner in which nucleic acids are disintegrated in the animal body.

So large a molecule can conceivably be disintegrated in many different ways; that is, the lines of cleavage might pass through several different points and in many different orders, but there is evidence available which causes us to believe that the process is quite constant in animal metabolism. Jones considers it probable that the first step is a decomposition of the tetranucleotid into dinucleotids, and that these are in turn split into mononucleotids. Little is known about the subsequent career of the two pyrimidine nucleotids, but we have an abundance of information concerning the nucleotids containing the purines, and it is in these our present interest lies. Each nucleotid has two points at which it might be split, and we have reason to believe that there exist in animal tissues enzymes which may specifically attack each bond. One enzyme separates the phosphoric acid radical from the nucleoside, thus:



and this enzyme is therefore designated as *phospho-nuclease*.

Another enzyme, *purine nuclease*, splits off, instead, the purine radical, thus:



Following either of these cleavages, the enzymes which deaminize purines begin to act, and we have formed as a result either the free oxypurines or the oxypurines still bound in the glucoside-like combination with sugar. If the purines are free the reaction will be:



or, in case the guanine glucoside is present:



In the latter case a hydrolytic enzyme, xanthosine-hydrolase, then splits off

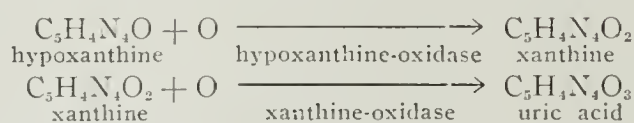
²Sitzungsber. k. Akad. Wissensch., Berlin, 1914, xxxiii, 205.

the xanthine, so that by either route the end result is the same. By a similar series of changes the adenine radical is converted into hypoxanthine, either directly by adenase:



or by adenosine-deaminase the hypoxanthine-glucoside (inosine) is formed, and later the hypoxanthine is split off.

We now have hypoxanthine and xanthine, which, in the presence of oxygen, are oxidized to form uric acid, thus:



Further oxidation of the uric acid causes its conversion into the much more soluble allantoin, thus:



It is thus evident that the steps of the disintegration of nucleic acid are numerous, but that each separate process is a simple one; and also, that it has been possible to follow out and distinguish the several steps and to establish the fact that each step depends on a distinct and specific enzyme. Not only has all this work been accomplished, especially by Jones and his associates, but, furthermore, these several enzymes have been studied in many different species and tissues, and found to have most peculiar and characteristic distribution. Our own work has been in this field, and I wish to recapitulate certain of the interesting facts that have been brought to light by different investigators of the biological distribution and significance of the enzymes of purine metabolism. The enzymes studied have been chiefly those that act on the free purines, for these are much more readily investigated than those acting on the nucleotids and nucleosides, and also they have been known for a longer time.

It has been found that not every tissue possesses all the enzymes of purine destruction, and also that in different species of animals the distribution of the enzymes is different. For example, the enzyme xanthine-oxidase, which oxidizes xanthine into uric acid, is found in man only in the liver, and also in other animals it is of limited distribution, being found usually only in the liver or in the liver and kidney, but in the dog it seems to be present in several tissues. The deaminizing enzymes, adenase and guanase, are much more widely distributed, but by no means universally. Adenase, for example, is not present in the tissues of the rat, and not in the tissues of adult human beings.³ Guanase is absent from the spleen and liver of the pig and from human spleen, although present in most other tissues. Uricase, the enzyme which destroys uric acid, also has peculiarities of distribution, being seldom found in any other tissue than the liver or kidney, and being absent entirely from the tissues of man, and from

³There have been some reports indicating the presence of adenase in fetal human tissues (Long, Jour. Biol. Chem., 1913, xv, p. 449).

the birds and reptiles so far examined. The significance of this distribution of uricase will be discussed at greater length a little later.

Now while it is unquestionably true that the results of laboratory experiments with tissue extracts cannot always be translated literally to the elucidation of metabolism in living animals, yet it has developed that the results thus obtained with purine enzymes fit remarkably well with results of metabolism studies, so that they are entitled to consideration and confidence. For example, the absence of uricase in man, birds and reptiles agrees perfectly with the fact that in the same animals we have much excretion of unchanged uric acid, whereas in all those animals that excrete little or no uric acid and much allantoin we find uricase. Furthermore, a striking correspondence is found in the absence of guanase from the liver and spleen of the pig and the occurrence of a form of gout in pigs, with deposition of guanine rather than of uric acid in the tissues.

Before taking up the bearing of these facts on human pathology, however, there are certain interesting observations on the general biological features of the purine enzymes to be mentioned. One of these is the appearance of these enzymes one by one in the development of the individual, and the general correlation of the order of their appearance with their distribution in the ascending scale of animal evolution.

To illustrate,—in the developing pig it was found by Mendel and Mitchell that nuclease was present in the earliest stages, adenase appears by the time the fetus has reached a length of 50 m.m., but xanthine-oxidase only appears at about the time of birth, while uricase appears somewhat later. So, too, in the human fetus we find guanase present by the third month, but xanthine-oxidase does not appear until at or near full term, and uricase never develops.

If we compare these observations with the occurrence of these same enzymes in organisms of varying stages of development in the biological scale we find a correlation that must be more than fortuitous. Thus, in a unicellular organism (yeast) there are found only nuclease, which seems to be present in all living cells, and also guanase; this corresponds to the early human or pig embryo, each with nuclease and one deaminizing enzyme. A mollusk was found to contain nuclease and both deaminases, corresponding to a later stage of mammalian development. The reptiles and birds are in the same stage of development as the mature human or pig fetus, being equipped with nuclease, adenase, guanase and xanthine-oxidase, but unable to destroy uric acid because of lack of uricase. Uricase seems to be a late development, acquired only by mammals, and not by all of them. On the basis of the facts cited above, and supported by others not mentioned, we may say that in the formation of purine enzymes we have an apparent exemplification of the biological law that the individual in its development recapitulates the development of its species—“Ontogeny recapitulates phylogeny.”

Studies in the occurrence of purine enzymes in tumors also give interesting results in general harmony with the statements made above. Both benign and malignant tumors are found generally to have the same purines and purine enzymes as the tissues from which they arise. Thus, human tumors contain universally nuclease, guanase, and enzymes which convert the bound adenine of adenosin into hypoxanthine by way of inosine, but agreeing with normal human

tissues, they contain no adenase which will deaminize free adenine. Furthermore, secondary tumors in the liver do not acquire the xanthine-oxidase which is present in human liver, but breed true to the parent cells. Tumors in the lower animals also present the enzymes characteristic of the corresponding tissues of these animals, at least to a certain extent, but they exhibit chemically their embryonal character as well as they manifest it microscopically. Thus, a primary carcinoma of the liver in sheep contained guanase and adenase, but no xanthine-oxidase or uricase; corresponding to the early fetal rather than to the adult sheep liver in lacking uricase and xanthine-oxidase. It is interesting to note also that in one instance we were able to obtain by an examination of the purine enzymes valuable evidence as to the origin of a certain tumor when histological evidence was inconclusive. This was in a rather common type of primary tumor of the sheep liver, which, on account of both its histological and gross characteristics has been believed by some to be of adrenal origin, that is, hepatic hypernephroma. In such tumors Long⁴ found adenase, an enzyme present in sheep livers but not present in either adrenal or kidneys of sheep, a fact which indicates that these tumors are not of adrenal or renal origin, but derived from the liver cells.

As yet we know nothing concerning the modifications which disease may produce in the purine enzymes, and the effects such modifications may have on metabolism. Quantitative studies of enzyme activity of tissues are seldom if ever of value, and we have no evidence that in any pathological conditions there occurs an absolute loss of any of the purine enzymes. On the contrary, I found that the liver of animals poisoned with phosphorus and hydrazine, so that a maximum degree of fatty degeneration was produced, showed quantitatively the same uricolytic activity as normal livers.^{4a} On the other hand, in liver tissue between cancer nodules there has seemed to be a decreased xanthine-oxidase activity; and in the bladder of a tuberculous monkey I found an appreciable amount of uric acid, whereas normal monkeys destroy practically all the uric acid and excrete little purine but allantoin.

As we have mentioned before, the human organism is peculiar in the lack of demonstrable uricolytic activity in any of its tissues. This fact has been established conclusively by the concordant results of several independent observers. The presence or absence of uricase is a very definite thing which permits of no possibility of error, for when present the action of uricase is strong and rapid. Thus, in an experiment with cat liver, it was found that this tissue destroyed completely one-twentieth of its weight (estimated as water-free tissue) in 2½ hours under laboratory conditions. Other livers were found fully as active. In view of the demonstrated presence of uricase in several mammals, it seemed desirable to determine as completely as possible just the distribution of this enzyme in nature. Studies of many different species did not reveal its presence outside the mammals. Among the mammals all the common domestic and laboratory animals, including also a marsupial, the opossum, showed uricase in some tissue, usually the liver.

On account of the peculiar exception of man in this respect, we secured tissues from different species of monkeys, and found that they too possessed

⁴Jour. Exp. Med., 1913, xviii, 512.

^{4a}Jour. Exper. Med., 1910, xii, 607.

active uricolytic enzymes in the liver tissue, thus resembling the lower mammals rather than man. At this stage of our work we were fortunately able to bridge the gap in our studies through the kind assistance of Dr. W. T. Hornaday, who placed at our disposal the body of a chimpanzee and also of an orang-utan in fresh condition. In each of these animals we found, as in man, a total lack of uricase.⁵ Hence these two anthropoid apes stand together with man apart from all other animals in their deficiency in uricolytic enzymes demonstrable by *in vitro* experiments. It is of particular interest that we find here the anthropoids resembling man, while the monkeys resemble the lower mammals, since this observation furnishes a new corroboration of other biological facts indicating that the anthropoids are more closely related to man than to the monkeys. And it is a striking fact that in this particular respect man and the anthropoids are less highly developed than all other mammals, for, as pointed out previously, the uricase is a late development in the animals that possess it, and apparently is the last acquisition in purine metabolism in the course of the development of the animal kingdom. So far as purine metabolism is concerned, man and the anthropoids rank in development with birds, reptiles, and some mammalian fetuses at about full term.

How well the results obtained by the examination of tissue extracts for purine enzymes agree with the metabolic characteristics of the several species of animals, is shown by the accompanying table prepared by Hunter and Givens.⁶

PER CENT OF PURINE-ALLANTOIN

Order and Species	Allantoin	Uric Acid	Bases	Uricolytic Index	Purine Co-efficient
MARSUPIALIA					
Opossum	76.0	19.0	6.0	79	4.1
RODENTIA					
Rabbit				95	26.0
Guinea-pig	91.0	6.0	3.0	94	27.0
Rat	93.7	3.7	2.7	96	37.0
UNGULATA					
Sheep	64.0	16.0	20.0	80	8.0
Goat	81.0	7.0	12.0	92	17.0
Cow	92.1	7.3	0.7	93	18.0
Horse	88.0	12.0	0.5	88	3.7
Pig	92.3	1.8	5.8	98	12.0
CARNIVORA					
Raccoon	92.6	5.4	2.0	95	16.0
Badger	96.9	1.9	1.2	98	28.0
Dog	97.1	1.9	1.3	98	29.0
Coyote	95.6	2.6	1.8	97	23.0
Cat				97	
PRIMATES					
Monkey	66.0	8.0	26.0	89	4.5
Chimpanzee				0	
Man	2.0	90.0	8.0	2 (?)	2.5

This demonstrates that all mammals as yet investigated, except only man and the anthropoids, excrete allantoin as the chief end product of their purine metabolism. The animals in whose tissues uricolytic enzymes have been found destroy nearly all the uric acid produced in metabolism, while the animals in which no uricase can be demonstrated show no capacity to destroy uric acid. Although human urine does contain minute quantities of allantoin this probably

⁵Jour. Biol. Chem., 1914, xviii, 157.

⁶Jour. Biol. Chem., 1914, xviii, 403.

does not represent a product of metabolism, for Ackroyd has found that ordinary foods contain traces of allantoin, and neither human nor other animal species seem able to destroy any allantoin they may form or receive preformed. Injected or ingested allantoin is excreted quantitatively in the urine.

As Jones has pointed out, the famous analysis of 10,000 liters of human urine by Kruger and Salomen yielded results that exactly agree with what might be expected from our knowledge of the location of the purine enzymes in the human body. They found that, besides the greatly predominating uric acid, the normal urine contained a little xanthine, somewhat less hypoxanthine, and much less adenine, the proportions being 10.1, 8.5 and 3.5, respectively; there was no guanine at all. Now since every human tissue has been found to contain guanase, the absence of guanine from the urine should be expected; and since guanase forms xanthine from guanine, and since xanthine-oxidase is present only in the liver, we should expect a considerable amount of the xanthine that is formed in the tissues to be excreted by the kidneys before reaching the liver, thus explaining the relatively high proportion of xanthine found in the urine. As the tissues in their metabolism can produce hypoxanthine from adenosine we should expect likewise a considerable amount of hypoxanthine in the urine, which was found. The small amount of adenine that was found also fits perfectly the fact that adenine cannot be destroyed in the human tissues, nor yet be formed from nucleic acid, hence a little adenine from the food should appear in the urine.

In view of all these striking agreements of fact and theory we believe that we are justified in applying the results of our *in vitro* experiments to the living organism, at least in part. We therefore seem entitled to say that the human organism cannot destroy uric acid, for it lacks uricase and excretes nearly all its purines as uric acid, and probably none as allantoin. In accord with this, several observers have found that uric acid injected into man is excreted quantitatively and unchanged in the urine. Furthermore, it has been shown that allantoin cannot be destroyed in the human body, and hence the nearly complete absence of allantoin in human urine seems to show that uric acid is not destroyed, at least by conversion into allantoin which seems to be the method used by all mammals that do destroy uric acid.⁷

But the question remains open: May not the human organism destroy uric acid by some other route than over allantoin. Evidence has been advanced in support of the hypothesis that at least part of the nucleoproteins of food can be destroyed by the human tissues, and, in view of the lack of evidence that human tissues can destroy uric acid it may be that some of the purines can be destroyed by some other route. Thus, free adenine cannot be destroyed by human tissues, yet when bound in the nucleoside, adenosine, as it occurs in nucleic acid, it is completely deaminized to hypoxanthine. We can easily imagine that bound purines may be disintegrated while still bound, and with the forma-

⁷Schittenhelm (Zeit. Exp. Med., 1914 (3) 397) who has consistently contended that uric acid is destroyed in the human body (in spite of the refutation of some of his experimental demonstrations of uricolytic activity in human tissues), has recently advanced another sort of evidence in favor of his main theme. He analyzed the lungs, liver, spleen and heart of a man who died after 6 days complete suppression of urine from bilateral thrombosis of the renal veins, and found all told but 0.01 g. uric acid. This finding is capable of the interpretation that uric acid can be destroyed in the human body, since otherwise an accumulation of uric acid should have occurred because of defective renal excretion. There are other possibilities to be considered, however, and the observation needs repetition to establish its value.

tion of some other end products than allantoin. A. E. Taylor,⁸ for example, has found that when the metabolism of a man is very completely studied and controlled, the ingestion of a certain amount of nucleic acid does not cause a corresponding increase in uric acid in the urine, although all the nitrogen of the nucleic acid is excreted. Other experimenters have obtained similar results. Apparently about half of the purine nitrogen thus administered is excreted in uric acid, but the other half as urea.

If uric acid formed in human metabolism cannot be destroyed, then part of the purines of nucleic acid taken in with the food in these experiments must have been disintegrated into something else than uric acid. That such a transformation of purines by an indirect route, avoiding uric acid, *may* take place must be admitted as possible, until either proved or disproved, neither of which has yet been done. There is evidence, however, that is not in favor of this hypothesis, and Siven⁹ has found that *B. Coli* will disintegrate purines in culture fluids, which makes it possible that the purine of ingested nucleic acid which does not reappear in the urine as uric acid, may have been destroyed by intestinal bacteria. Divergent results in feeding experiments may be explained thus by differences in the character of the intestinal flora and the rate of absorption. Further, the studies in Müller's clinic on the metabolism of the nucleosides,¹⁰ adenosin and guanosin, show that both in normal and gouty individuals the purines of these nucleosides are excreted as uric acid, which indicates that they are not disintegrated by some special route which avoids the formation of uric acid. Also in leukemia and pneumonia we find evidence that the purines liberated from the disintegrated nuclei are excreted as uric acid, and no evidence of other transformations. It is, indeed, improbable that one mammal should have a special method of destroying uric acid unknown in any other mammal, especially in view of the identity of other processes of purine metabolism all through the animal kingdom.

We may therefore state the case of uric acid in human metabolism to stand at present as follows:

(1) There is conclusive evidence that the human organism does not oxidize uric acid formed in metabolism into allantoin, as all other mammals except the anthropoids seem to do. (2) There is no conclusive evidence that man destroys uric acid by any other method, although it has not yet been positively established that he may not do so. (3) There is perhaps more reason to suspect that part of the purines of nucleic acid may be destroyed without passing through the stage of uric acid, but there is no direct evidence whatever that such a method of purine katabolism does exist. (4) It is highly probable, in consideration of what seems to be the best available evidence, that most of the purine of human food and practically all the purine from cell metabolism is converted into uric acid and excreted as such. Some of the food purines may be destroyed by bacteria in the intestines.

⁸Tour. Biol. Chem., 1913, xiv, 419.

⁹Arch. ges. Physiol., 1914, clvii, 582.

¹⁰Zeit. physiol. Chem., 1914 (91) 336.

THE FERMENT-ANTIFERMENT BALANCE OF THE SERUM*

BY JAS. W. JOBLING, M.D., WILLIAM PETERSEN, M.D., AND A. A. EGGSTEIN, M.D.,
NASHVILLE, TENN.

AMONG the numerous chemical and physico-chemical systems of balanced reactions that occur in the animal organism few offer a more interesting field of investigation than that existing between the proteolytic serum ferments on the one hand and the serum antiferment on the other. Interesting not only to the physiologist in its influence on the normal metabolism but especially to the pathologist because of the fundamental importance which the whole domain of protein intoxication has assumed in the recent development of the study of pathological problems.

For the clear conception of the potential toxicity of the protein molecule we are in debt to the work of Vaughan in this country and to Pfeiffer, Friedberger, Kraus, Zunz and numerous other investigators in Europe, as a result of whose work we now know that the split products of proteins, especially the larger fragments, are toxic no matter what their source; that no well defined "antitoxin" is formed following injection of such split products, but that the protein fragments themselves, on further hydrolysis, become nontoxic. Under these circumstances we should expect that the presence of proteolytic ferments in the serum would lead to an immediate intoxication of the animal. To prevent such an untoward result the blood has been provided with an antiferment.

Our knowledge concerning both serum protease and antiprotease has been limited in the past, due possibly to the fact that effect rather than cause has been studied by most workers, while of the two factors in the balance, the antiferment has received the greater share of attention largely because of certain alterations in its titre which were observed clinically.

SERUM ANTIFERMENT.

Three theories have been formulated concerning the nature of the antiferment. It was regarded at first as a true antibody similar to those obtained when toxins are injected into an organism; the secretions from the leucocytes or the pancreas being considered the source of a constantly stimulating antigen. This position is, however, not only not physiological but the experimental evidence brought against it is so complete that it has been abandoned. The supposition was next advanced that the antiferment action was due to the accumulated split products of proteins in the serum which checked further enzyme activity. It was found, however, that the experimental evidence presented by Rosenthal in advancing this theory was incorrect. Among the first to suspect that the serum lipoids might have some relation to the antiferment property was Schwartz. Several serious objections were immediately advanced against this idea: the extracted lipoids appeared to be inactive; lipid rich sera were not necessarily more strongly antitryptic than normal sera. Our interest in the subject was based on observations made during the course of studies concerning the

*From the Department of Pathology, Vanderbilt University Medical School.

mechanism of caseation in tuberculosis. In that process we determined that the lipoids, probably derived largely from the tubercle bacilli, completely inhibited tryptic digestion, thus accounting for the lack of autolysis in the diseased foci. When such lipoids were saturated, as with iodine, the inhibitory effect was lost. In order to study the lipoids to greater advantage we saponified various preparations, in this manner greatly increasing the dispersion. It was found that the antiferment property was proportional to the degree of unsaturation of the soaps.

In the serum itself it had previously been observed that the lipid solvents—ether, chloroform, etc.—removed or destroyed the antiferment, and Opie had noted that dispersion changes, such as are induced by acidifying the serum, rendered it inactive. Heating the serum to about 70° C. for 30 minutes has the same effect. On closer investigation we found that the extracted lipoids of the serum were actively antitryptic if their degree of dispersion was brought back to approximately its original state, as for instance by saponifying the lipoidal residue. We were able to demonstrate then that (1) the antiferment property of the serum depended upon the lipoidal constituents, whose activity depended on the degree of unsaturation and the degree of dispersion; (2) the antiferment was extractable in lipid solvents, (3) the residue was actively antitryptic when the dispersion was again brought back to approximately the original state.

THE SERUM PROTEASE.

The nonspecific serum protease has been studied very little. Hedin had observed that ox serum contained a weak proteolytic ferment, associated with the globulin fraction of the serum protein. Delezenne and Pozerski had previously made the interesting observation that serum treated with chloroform became actively proteolytic when incubated, but found no explanation for the phenomenon; Von Dungern and Hirschfeld, too, had observed that serum treated with iodine underwent autolysis. Later, Opie considerably extended the studies commenced by Hedin.

All this work was of fundamental importance, but the facts determined remained isolated and without connection. As to the relation between the ferment and antiferment, Hedin rather considered the combination as a true pro-enzyme or zymogen, indeed typical for such a stage of the ferment, regarding the activation of the ferment merely as a process by means of which the anti-enzyme was destroyed.

On investigation we determined that the protease is present in all guinea-pig sera, usually in dogs and rabbits, rarely in human sera. It is usually inactivated when the serum is heated to 56° for 30 minutes, although occasional heating to 60° for 30 minutes will not inactivate the protease completely. Its activity is best demonstrable in a neutral or slightly acid reaction; it retains its activity unimpaired when dried, and for a considerable period of time when the fresh serum is placed in the ice chest. It is polyvalent in character, but bears no relation to the leucocytes.

SEROTOXIN, ANAPHYLATOXIN AND THE MECHANISM OF ANAPHYLATIC SHOCK

If we now investigate a serum which under normal conditions contains considerable ferment, such as that of the guinea-pig, and determine the changes that occur when the antiferment is removed, several rather interesting phenomena

are observed. The serum becomes highly toxic for its own species. Thus one sample, incubated with chloroform for twenty-four hours became so toxic that 0.3 c.c. were sufficient to kill a medium size guinea-pig almost instantly. On further incubation the toxicity decreases, while a progressive increase in non-coagulable nitrogen can be observed during the course of the incubation.

The symptoms in the injected animals closely simulate anaphylatic shock. Following recovery from a sublethal dose there results a moderate increase in resistance to reinjection, similar in extent to that which Zinsser and Dwyer have observed following injection of anaphylatoxins.

It is well known that there has existed for several years considerable doubt as to the source of the toxic material of the so-called "anaphylatoxins." According to Friedberger and his school, the fresh serum digests the antigen, which is added in the form of bacteria, precipitates, corpuscles, etc., with a resulting splitting of the antigen to toxic split products. Opposed to this view we find arrayed the physical hypothesis which Ritz and Sachs, Doerr, Bordet and others have formulated. It was observed that quite inert substances, such as kaolin, barium sulphate, agar and starch would, when incubated with the serum, render the serum toxic; quite obviously split products, if responsible for the toxicity must be derived from the serum itself. Nor has it been clearly demonstrated that a splitting occurs when the bacteria are treated with fresh guinea-pig serum. Donati, for instance, found the bacteria intact as far as antigenic properties were concerned, while Neufeld and Dold noted that bacteria which did not undergo lysis by the serum were more efficacious than those which dissolved during anaphylatoxin formation.

In view of the rapid development of the toxicity of guinea-pig serum when its anti ferment is removed by chloroform we quite naturally were justified in assuming that during typical anaphylatoxin formation adsorption changes occur by means of which the serum anti ferment is adsorbed and the serum proteins themselves hydrolyzed, rather than the introduced antigen. From a purely physical viewpoint this is quite rational, for the lipoids in general and the fatty acids more particularly are easily adsorbable substances. Our investigation showed this hypothesis to be substantially correct, and it has been recently confirmed. We found that the anti ferment was adsorbed by the bacteria; that the noncoagulable nitrogen increased, and that the bacteria, because of adsorption of anti ferment, became more resistant to tryptic digestion.

If such conditions explained the toxicity of the anaphylatoxin, might it not be possible that a similar mechanism occurred during anaphylatic shock? In order to carry out these experiments we used dogs exclusively, because of the larger amounts of serum needed. We found that following the first or sensitizing intravenous injection of the antigen there were practically no serum changes as far as the ferments or anti ferments are concerned. When, however, a reinjection is made after the animal is fully sensitized the following changes were noted immediately after the injection: The anti ferment was markedly diminished; there was a striking increase in serum protease and lipase; an increase in noncoagulable nitrogen and amino nitrogen; a decrease in serum proteoses. The increase in amino acids might be interpreted as proof of the fact that hydrolysis of the antigen had occurred, as Zunz and György maintain, but under such circumstances we should expect that there would also be an increase in the

higher split products, such as the serum proteoses. This does not obtain. We are rather inclined to place the emphasis on the change between the ferment-antiferment balance which here greatly favors proteolysis. Under such circumstances it seems more probable that it is the serum protein itself which is hydrolyzed to toxic products and that the injected antigen initiates the process by causing the instantaneous mobilization of the nonspecific protease, together with changes in the colloidal dispersion whereby the serum lipoids become less active as antiferments.

CONCERNING THE MECHANISM OF THE ABDERHALDEN REACTION.

It is quite apparent from the preceding work that the serum itself, will, under proper conditions, hydrolyze and yield split products. Keeping this in mind, the repeated observations that a positive Abderhalden reaction can be obtained when quite inert substances are incubated with serum, such as kaolin, agar, etc., would naturally lead us to suspect that the same simple absorption phenomenon is at the basis of the Abderhalden Test. We do not believe that the specificity of the reaction in regard to protease action can longer be maintained in view of the adverse clinical findings reported during the past two years. Almost every febrile case will give a positive Abderhalden reaction against placenta at some time during its course, as Falls, among others, has shown. It has been noted, however, that during experimental immunization or sensitization a reaction is obtained which is seemingly specific. This we believe can be easily explained by the colloidal changes (precipitation—adsorption) which are induced in such sera when the antigen is added to them. The antiferment is diminished and in the local areas of deficiency so formed the serum proteases and ereptases can become active and digest the serum proteins with a resulting production of amino acids and a positive Abderhalden Test. We rather believe that the Abderhalden Reaction, while it has been of considerable value in calling attention to the importance of serum ferments, has really added unnecessary complication by the confusion of the ordinary immunity reactions with proteolytic cleavage supposed to be specific. As a result the normal polyvalent serum protease has been neglected.

SERUM PROTEASE FOLLOWING INJURY BY BURNS, HEMOLYSINS, PHOTODYNAMIC SUBSTANCES, ETC.

Probably the only recent work has been that of Pfeiffer who has used glycytryphthophan as an indicator of peptolytic activity. During the course of his studies he has noted that following injury, as for instance by burns, a great amount of ferment is thrown into the serum, which Pfeiffer considers as an evidence of cellular destruction, believing that the ferment is derived only from cells that are destroyed. He has noted the same increase after the injection of hemolysins, during photodynamic intoxication and also during anaphylatic shock. We do not believe that it is necessary to assume total destruction of cells before ferments are liberated, but rather that such a mobilization can occur from an injury which need not end in total destruction of the cell. In other words, the mobilization of the ferments can be regarded to some extent as a protective cellular reaction rather than as an evidence of cellular destruction.

CHANGES DURING LOBAR PNEUMONIA.

This seems to be made evident by the conditions that are found to prevail during the course of a typical lobar pneumonia. It had been observed previously that a rapid increase in the serum antiferment occurs during the course of the early stages of the disease with a decline at or just after the crisis. In a study of a number of cases we have noted that at the time of the crisis a well defined increase in serum protease occurs which again disappears as soon as the crisis is passed. We can regard the serum changes either as a reflection of the processes taking place in the involved area, or as primary changes which influence the pathological processes. Considering the intimate relation known to exist between the inception of autolysis in the involved lung and the recovery of the patient it would seem reasonable that these two observed factors—a lowering of the antiferment titre and the increase in the amount of protease—are of importance in initiating the autolytic process in the lung, and thereby the onset of the crisis. In this explanation we of course assume that the source of the toxic substances are not to be sought solely in the pneumococcus protein, but that the pneumonic exudate itself, consisting of a tremendous amount of fibrin and leucocytic debris, a foreign mass in so far as the lung is concerned, represents a matrix of potentially toxic substances. As long as the inhibitory factors are in the ascendancy the digestion of this material must proceed slowly, so that only the higher toxic split products are absorbed. As soon as the inhibitory factors are overcome, however, active autolysis can begin, the lower split products only will be absorbed and recovery can take place. Falls has recently demonstrated an increase in proteolytic serum ferments at the time of the crisis by means of the usual Abderhalden technique. Such a ferment increase might be interpreted as having its origin in the leucocytes of the involved lung tissue as Falls states, rather than being considered a reaction product of the cells as a whole. Were this the case we should expect that the ferment would continue to be present for a considerable time after the crisis, when autolysis is rapidly clearing the lung tissue. This, however, is not found, for after the crisis the protease almost immediately drops back to its original titre. Falls has not been able to find any relation between the leucocyte curve and the protease content of the serum, nor have we noted any connection. It would therefore seem reasonable to suppose that the serum protease does not take origin from the leucocytes. Nor is its source the pancreas, for when the organ is excised, the animal responds with a mobilization of protease on intoxication just as it would normally.

BACTERIOTHERAPY.

Our interest centered naturally about studies of the ferments following bacterial injections. As a result of the work that has originated through the study of the Abderhalden reaction, numerous efforts have been made to obtain specific ferments against bacteria, with results quite at variance. It is of course well known that bacteria are resistant, both when living and after being killed in various ways, to the most powerful proteolytic ferments, a resistance due most probably, we are inclined to believe, to the fact that the limiting membrane of the organism is largely lipoidal in character. We have found that various dried organisms are resistant to digestion almost in proportion to the amount of unsaturated lipoids contained.

While therefore no effect of the strong polyvalent tryptic ferments have been observed, Abderhalden and various workers have endeavored to show that specific ferments might be obtained that would digest the bacteria. The evidence for this assumption is quite contradictory, and rests, we believe, on the ordinary immunity reactions. The whole subject is important from the therapeutic point of view, especially because of its bearing on vaccines and bacteriotherapy in general.

As a result of our studies we have observed that the intravenous injection of dried organisms is followed by a very rapid mobilization of serum protease and lipase, the increase being proportional in general to the toxicity of the organism, and its resistance to tryptic digestion. Thus following an injection of 10 mg. of dried typhoid bacteria in a dog the proteolytic activity of the serum increased from an original titre of 0.1 mg. (the amount of noncoagulable nitrogen formed from 1 c.c. of serum under chloroform in about 18 hours) to 0.78 mg., five hours after the injection; the lipase titre increasing at the same time from about 1 c.c. N/100 NaOH (the acidity produced from 1 c.c. ethyl butyrate by 1 c.c. serum in 4 hours) to 6.5 c.c. N/100 NaOH. With tubercle bacilli on the other hand, which are resistant to digestion, practically no changes were noted in the serum ferments.

It is a well recognized fact, admitted even by Wright, that the therapeutic results following the injection of bacteria (vaccines) are not to be explained wholly on a specific basis, certain facts indicating that nonspecific benefits result from bacteriotherapy. The possibility that the explanation for this effect is to be sought in the mobilization of nonspecific ferments, such as we have demonstrated following bacterial injection, must be kept in mind.

This subject has assumed a more than casual interest in view of the numerous reports of the successful use of vaccines administered intravenously during the course of typhoid fever. Inasmuch as antibodies are being formed with considerable constancy during the disease under the usual conditions, there could, theoretically, be no object in seeking to stimulate a function of the cells already producing these substances to their full capacity. And the recent reports of almost immediate recovery following the intravenous injections of vaccines have emphasized the fact that following such injections there are practically no changes in the antibody concentration. This would seem to be in line with the understanding reached in late years that recovery in typhoid fever cannot be explained wholly on the basis of the production of the ordinary antibodies, for the patient may indeed reach a high antibody concentration long before the disease process has been checked. The observation of Kraus, among others, that the defervescence occurs as well after an injection of colon vaccine in typhoid fever, and of Ishawara, who aborted paratyphoid infections with typhoid vaccines, leaves no room for doubt that the idea of specificity is to be excluded in the mechanism and that an explanation must be sought along other lines.

In this connection it should be pointed out that there are certain objectionable features to the bacteriotherapy as used in typhoid fever, objections inherent in the toxicity of the organisms and only partially overcome by the treatment with serum such as used in the preparation of the Besredka vaccine. These concern chiefly the effect on the circulatory apparatus, with occasional evidences of a grave collapse, and the increase in peristalsis.

If we regard the therapy as one designed to stimulate a mobilization of non-specific ferments it might be possible to elicit such changes without the employment of an agent itself toxic. To this end we have studied the effect of the intravenous injection of the various split products of proteins, using several proteose and peptone solutions. As Jobling and Strause and Zunz have shown, the secondary proteoses are much less toxic than the primary, the use of such solutions might be of value if the stimulation is sufficient. Lüdke has worked along a similar line and reports very favorable results when solutions of deuteroalbumoses are injected intravenously during the course of typhoid fever.

THE EFFECT OF A CHILL ON THE ANTIFERMENT.

The injection of the vaccines as well as the deuteroalbumose solutions is followed clinically by a marked chill, and the serum examination usually shows a well defined reduction in antiferment titre immediately after the chill. Experimentally a similar phenomenon is noted when guinea-pigs, for instance, are chilled for a period of about one-half hour. Thus in one animal the antiferment titre fell from 59% to 0.0% per 0.075 c.c. of serum, in another from 75% to 59%. Inasmuch as the antiferment consists of highly dispersed lipoids, it seems reasonable that during the greatly increased metabolic demands incident to the chilling of the animals, the lipoids would be used as available material. We therefore feel justified in ascribing the lowering of the titre following the vaccine injection to a similar cause. This fact in conjunction with the possible increase in serum protease would of course greatly facilitate proteolytic processes.

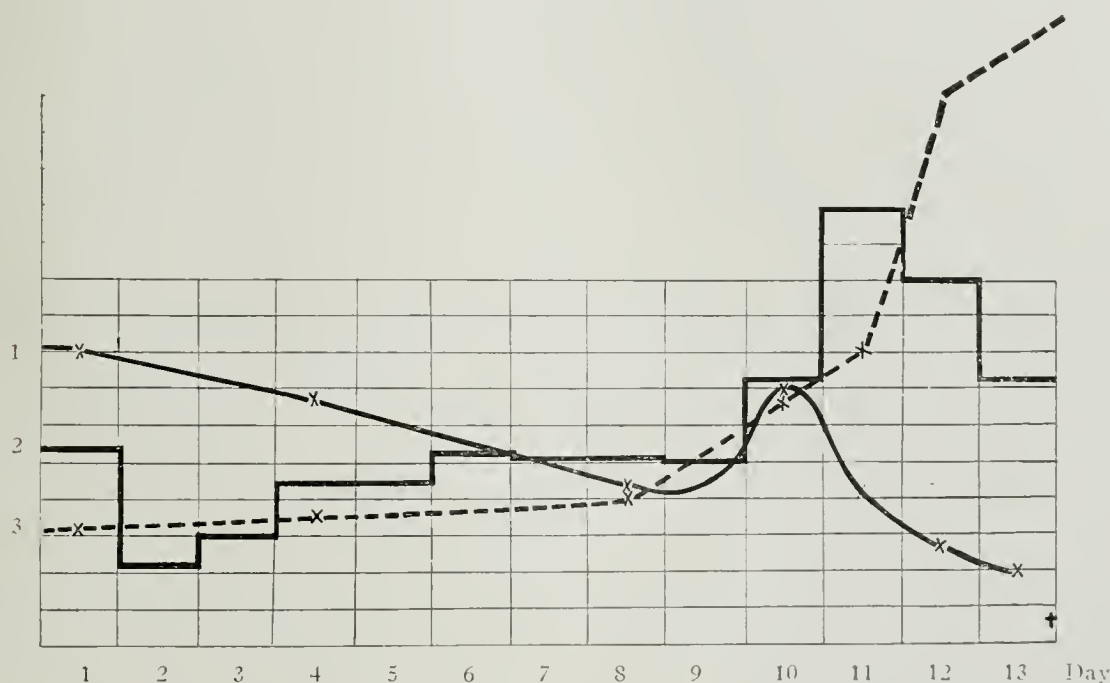
THE CAUSE OF DEATH FROM INANITION.

The lowering of the antiferment titre due to a similar metabolic demand and involving an interesting form of true auto-intoxication, is that which occurs during starvation. It is of course a well known phenomenon that a large increase in nitrogen excretion occurs just before death in starving animals,—the so-called premortal rise,—while the animals themselves, during the course of the experiment, present definite evidences of intoxication, to such an extent that Schulz has advanced the idea that during starvation the body metabolism is so altered that periods of intoxication occur at intervals, culminating in the death of the animal from such a cause. Schulz's views are contrary to those of Voit, who connects the death of the animal with the exhaustion of the fat depots of the body. In studies which we have made we have determined the following facts: During the course of the experiment a definite and progressive lowering of the antiferment titre occurs, a minimum being reached just before the premortal excretion of nitrogen. At this time the noncoagulable nitrogen of the serum increases very markedly and the serum protease overbalances the inhibitory effect. A typical protein intoxication occurs, with an increase in antiferment following as a reaction. If sufficient to check the process the animal may recover and live over an additional period of several days; if not, death ensues rather promptly. The death is therefore not of necessity due to a lack of available energy material, but to an intoxication due to protein split products formed when the antiferment has been reduced to such an extent that the ferment overbalances it. If the antiferment is artificially increased the animal may be tided over such periods of intoxication, just as we have shown that the antiferment may afford some protection against anaphylatic shock.

An illustrative experiment is shown in the accompanying chart.

Curve 1 represents the antiferment titre in per cent of inhibition per 0.075 c.c. serum. Curve 2 represents the daily total nitrogen excretion in tenths grams. Curve 3 represents the total noncoagulable nitrogen per c.c. of serum in tenths milligrams.

It will be observed that the premortal rise in nitrogen excretion began in this rabbit on the tenth day. The changes in the ferment-antiferment balance had occurred the day previously, i. e., on the ninth day, when the antiferment titre reached its lowest level. As a result an intoxication occurred, evidenced by the progressive increase in noncoagulable nitrogen and the increase in antiferment for a short period. The animal died on the thirteenth day of the experiment.



THE THERAPEUTIC EFFECT OF IODIN.

The lowering of the antiferment titre and the resulting aid to proteolytic processes need not of necessity lead to disastrous effects, indeed it seems that it had been the basis of the mechanism by means of which iodine has been of great value therapeutically.

In studying a series of cases placed at our disposal through the courtesy of Dr. Fordyce and his associates, we have noticed that under a course of iodides the antiferment titre is quite constantly diminished. Whenever symptoms of iodism occurred, the titre again increased to its former or greater value. Experimentally it has been shown previously that iodides and iodine increase the autolysis of macerated organs, but when injected into animals the effect on the antiferment has as a rule been an increase. This latter fact is most probably due to the large doses used and to the fact that practically all experimental animals have a low titre to begin with as compared to human sera, so that changes are much less pronounced.

The manner in which the iodine lowers the antiferment titre is we believe rather simple. The antiferment property, depending on the unsaturated bonds of the lipoids, is diminished by any agent which tends to decrease the unsaturation of these bodies. Iodine and iodides given over a period of time gradually are taken up by the lipoids and saturate these valences with a resulting less-

sening of the antiferment property. Whatever proteolytic ferments are then present will of course have a greater field of action and necrotic tissue especially will undergo autolysis and disappear. This explanation offers a reasonable basis for an empiric therapy which is of undoubted value in the removal of necrotic material; in the exposure of diseased foci in necrotic masses to the effect of specific therapeutic agents; and possibly in the treatment of infectious diseases when greater proteolytic activity is to be obtained.

THE INCREASE IN ANTIFERMENT.

That an increase in antiferment would be of value in preventing the formation of protein split products and thereby act as a protective agent is beyond question. There seems to be evidence however, that an increase in the antiferment may perceptibly alter the metabolism of the organism, as for instance in bringing about the condition of hibernation. Theoretically it should be possible to increase both the intracellular as well as the serum antiferment to such an extent that protein cleavage of any kind would be held in abeyance.

During the course of our work we have noticed that even in dogs the antiferment titre seems much lower during the summer than the winter, the decrease in some instances amounting to fully fifty per cent. Probably the work of Mayer and Schaeffer is suggestive in this connection. By means of careful quantitative studies they noted that there was not only an increase in the relative amount of lipoids of the serum and tissues during hibernation but the iodine value of the lipoids was greatly increased, indicating an augmented antiferment power. Whether this bears some relation to the peculiar glandular enlargements noted during hibernation has not been determined. The subject is one of several in which the direct influence of the antiferment seems apparent. The field is so broad and the applications so many that we have simply endeavored to indicate a few lines along which the relative changes between ferment and antiferment have seemed to us to play a part in the mechanism of pathological and physiological processes.

A complete review of the literature is found in a series of papers published in the *Journal of Experimental Medicine*.

EXPERIMENTAL AND CLINICAL STUDIES ON MENTAL DEFECTIVES, I.

A PRACTICAL SYSTEM OF DIAGNOSIS AND STUDY BASED ON THE PRINCIPLE OF GROWTH-DEVELOPMENT AS THE EXPRESSION OF CONSTITUTIONAL CHARACTER OR DEFECT.

BY AMOS W. PETERS, PH.D., WITH THE ASSISTANCE OF CAROLINE D. BLACKBURN,
PHILADELPHIA, PENN.

THE view of the pathology of the feeble-minded which is expressed in the above title is the outgrowth of several years of combined laboratory and clinical study of such cases. The technic here described is designed for the examination and study of the mental defective from the physio-pathological as

well as the psycho-pathological standpoint. I believe that the evidence of studies made by this technic shows that with mental defectives including the feeble-minded we are studying primarily errors of physical growth and development whose essential nature is not simply neural but extends in some degree, and in many cases most pronouncedly, to any or all other systems of organs. This practical generality of pathological condition we may justly ascribe to intimate hormonal or neural correlation or both. In the majority of cases these deviations from the normal course are constitutional, i. e., they are inherent in the character of the germ cells, they begin with the parentage, are continued and magnified during the period of gestation, and are deeply impressed in the physical constitution of the individual so that abnormal growth and development characterizes such individual not only during gestation but continuously after birth. The acquired cases differ from the others only in the fact that the *incidence* of the disturbance of their growth-development has occurred at a later period in the cycle of development. The fundamental fact even in these cases is still the perversion of the normal course of growth and development of the physique.

The feeble-minded, therefore, stand before us as a biological, a pathological phenomenon, the physio-pathological study of which can be differentiated into a physiological and a biochemical subdivision. It should be noted that the pursuit of this problem by any one physiological or pathological method exclusive of the others is fatal to the success of that one method itself. As subsequent publication of our study of these cases will show, this fact is due to the complexity of these pathological conditions, which greatly exceeds that of the current conception of the simple omission of a something from their neural development. Having begun with an effort to further the solution of the pathological problem of the feeble-minded by the immediate application of the biochemical method, it gradually became evident that for intelligent orientation and interpretation this method stood in need of a generalized viewpoint to guide its attacks. Experience demonstrated that such study should be preceded or at least accompanied by a preliminary introductory examination of an anatomico-physiological nature. It was thus found necessary to evaluate the physique physiologically and pathologically, upon which experiment by the biochemical method was to follow. We have therefore described our technic in its logical order, thus deferring the biochemical parts to subsequent papers. For the purpose of this preliminary orientation the system of procedures below described was evolved from the practical examination and study of sufficiently numerous cases and this method is now used as an introductory and diagnostic routine for subsequent more intensive studies as shown below in the general plan. Its utility and application is by no means confined to a first examination. The development of this system has placed the writer in the possession of the results so obtained on fifty miscellaneous cases comprising eighty-two examinations which include both normal and pathological individuals. An interesting part of this material consists of several family groups in which are comprised both psychologically normal and psychopathic members of the same family. These results will be subsequently reported.

The utilities of the following orderly system of examination and study may

be summarized as follows. *First*, stands the immediate diagnostic value of the results thus obtained. *Second*, the observation and measurement of growth-developmental changes as they occur in the natural course of physiological development of both defective and of normal subjects, and the resulting possibility of accurate comparison with the growth-development of normal individuals. *Third*, the measurement of growth-developmental changes as a measure of the results obtained by any given method of care and treatment, whether the conditions be physiological, pathological or experimental. *Fourth*, the study of the inheritance of the characters involved, or shown by a comparison of the results obtained on family groups.

In this connection we may record our experience, that because the technic here described touches the personality of the subject in such manifold ways, a competent and experienced examiner will successfully diagnose from 75 to 100 per cent of unselected mental defectives independently of the customary psychological examinations. This is not to be taken as an argument or justification for the omission of the latter, for our plan of investigation definitely provides for an examination of the mental ability of the subject. However, the above success which we have repeatedly observed does show how intimate and evident the correlation between physiological and psychological conditions is in these cases.

It should be noted that the same technic is intended to serve the important purpose of making a parallel survey of mentally normal subjects not only for comparison with the defective but also for the study in the human subject of normal growth and development itself. The importance of such surveys of normal individuals for both scientific and practical purposes should not be underestimated. The collective data on well selected normal material are too few in the literature, the results that might thus be obtained are of evident importance, and the experience to the examiner of noting certain contrasts in the results obtained on normal and defective subjects, are considerations that should induce one to make use of every opportunity that offers for obtaining data on any physically peculiar but mentally normal person whether child or adult.

We are confronted at the outset with the practical question as to which examination we should make first, the physio-pathological or the psycho-pathological. Customary practice thus far scarcely takes cognizance of the practical significance of anything beyond the psychological examination. Physical examinations it is true are made as an almost meaningless routine, for they are not correlated with the mental findings, are not incorporated as related integrals into the ensemble of a complete examination, and they are too often made only from the physician's customary standpoint of the temporary health of the subject and thus completely fail to seize those data which pertain to the growth-developmental defects which are the most fundamentally important facts in the pathology of the case. In our opinion the systematic survey should begin with the physiological examination rather than the psychological. Experience will teach the examiner that the present physique of the subject, his physio-pathological history, the history of his growth and development and the ascertainable facts of his heredity provide a logical background for the psychological findings. The direction of logical explanation is from the physiological condition as a causa-

tive basis to the psychological as an intelligible effect and not the reverse, especially in the case of mental defectives, regardless of what opinion one may hold for certain other psychiatric cases or conditions. To interpret the psychological findings or in the present state of our knowledge even to make an attempt to explain them, the underlying physio-pathological conditions must be recognized to be the determining substratum. The reverse procedure may possibly some day become practicable when our knowledge shall have increased far beyond its present bounds. To the practical examiner who comes in contact with such cases as will be subsequently described in these papers and who accustoms himself to place in juxtaposition both physiological and psychological findings the above arguments are superfluous. Our purpose is rather to emphasize the necessity of actually making the right kind of physio-pathological examination and especially of practically recognizing the pathogenetic relation between the physical and the mental findings.

At this point we desire to guard against certain misconceptions which are probably due to use of the term "examination." The investigation of a subject as here planned is rather a study, the preliminary and introductory parts of which may be made at a single initial conference occupying from two to three hours or more depending upon the findings. The kind of examination here described involves careful observation, and especially experimental physiological testing with which hasty and routine procedures are wholly incompatible. The examiner needs an assistant both for the procedures themselves and for recording from dictation the measurements and observations or two examiners can profitably work together. The writer working under institution conditions permits two women assistants to do as much of the preliminary examination as they are trained for and accustomed to do but makes it a rule to survey each new case carefully himself at the close of the introductory examination and then dictates his own observations as personal "addenda" to the record already made. This saves the examiner's time without depriving him of an increasing and very desirable first-hand experience. The form of record should be so flexible and adjustable as to permit extended description where the conditions indicate its value. The worst form of record is that which provides a fixed and usually small space for the same heading for all cases. This form encourages routine expression and omission. It will happen that a heading in some cases will require extended description, in others only the word "negative" or "normal." We therefore adopt the plan of having a printed or typewritten outline before us as a guide in which each point to be noted is given an unchangeable number by which it is represented in the written record thus permitting the space for each heading to be adjusted to the greater or lesser extent of the findings in each case. Great care should be taken to preserve in systematic way the original records which are made in the presence of the case and to distinguish between these and any copies or elaborations subsequently made. The trained laboratory worker, of course, always observes this distinction and rigidly refrains from substituting a "copied" record which is "nicer" or "better" for the original made in the presence of the facts themselves. The observer should if necessary train himself away from the duplication of labor and the experimental error involved in copying first records of observations for

the purpose of improving them. The disorder thus sought to be remedied must not be permitted in the first instance. All records must bear *prima facie* evidence as to who made the observations and when they were made.

It should be emphasized that the utility of the present plan of examination and study is by no means confined to a first or preliminary investigation. A study of the progressive growth-developmental changes of a young person or of the progress of certain pathological changes can be made by repeating at proper intervals selected portions of the plan of measurements and of observations and tests. It is surprising for what short intervals exact measurements can demonstrate growth in a rapidly developing young subject as some repeated measurements recorded by this method show. An accurate record of such measurements and observations on development taken on the same individuals of both normal and of mentally defective types would be highly interesting and detailed data on the history of the same individual are exceedingly rare if not entirely absent from the literature. On the other hand there are considerations which make a certain amount of repetition of measurements when the subject is not perceptibly changing of importance. Such repetitions made by the same and by different examiners give us the data for estimating the limits of variability of the method itself either in the hands of the same or of several different examiners. It will also soon become apparent to the practical worker that some items in the scheme of measurements are capable of much more exact repetition than others and he should have some conception of the amount of the variability for each measurement. To this subject we shall return later in the discussion of some of the results obtained.

We now come to the practical question as to what should be included in an adequate scheme of examination and diagnosis of the mental defective which scheme should not stop at this point but also furnish the basis for further study and research. The content of such a scheme will be much influenced by two factors one of which is the author's views on the possible pathogenesis of such cases and the other is the present state of our knowledge or better, the present degree of development of the method and technic of scientific investigation. After having studied the pathology of these cases for a number of years and having made what must of course be only a personal survey of current scientific method that is available or that can be applied to the problem, the writer ventures to propose for consideration the following subdivisions of such a scheme:

GENERAL PLAN FOR THE STUDY, DIAGNOSIS AND INVESTIGATION OF MENTAL DEFECTIVES

- A. Anthropometric measurements.
- B. Physio-pathological observations and tests, including data on abnormalities of growth-development.
- C. Physiological and pathological history of the individual.
- D. Family history and especially data pertaining to the mother during the period of gestation.
- E. Mental, and especially intelligence, tests and studies of the individual both independently and in the light of the preceding data.
- F. Experimental physiological and biochemical evaluation of the pathology of the individual, especially studies on the metabolism and blood.

G. Experimental care-taking of feeble-minded individuals or of small groups for the *application* of all the available scientific data above obtained, or obtainable in scientific literature, to the *physical improvement* of these subjects, including especially their diet and nutrition.

H. Anatomical, histological and biochemical studies on the organs and tissues of the individual, especially on the nervous and the glandular systems, inclusive of glands of internal secretion.

Armed with a foundation of pathological knowledge we could now add the logically following topics of prevention, therapeutics if any, personal care and management of these cases, and finally the most scientific solution of the sociological problem of the mentally defective.

OUTLINE FOR THE EXAMINATION AND STUDY OF MENTAL DEFECTIVES

DIVISION A. LIST OF ANTHROPOMETRIC DIMENSIONS.

Group I: Some General Dimensions. See Notes Nos. 2-14.

1. Age = A. 2. Weight = W. 3. Total height = H. 4. Arm span = S. 5. Height to lower margin of symphysis pubis = Sy. 6. Height to navel = Na. 7. Height to iliac crest, Right, Left = I.

Group II: Thoracic Dimensions. See Notes Nos. 2-14.

8. Smallest neck circumference. 9. Total basal profile = Bf. 10. Sections of basal profile, Bf1, Bf2, Bf3. 10a. Drawing of basal profile. 11. Basal line = (Friedenthal's a) = B. 12. Transverse shoulder line = (Friedenthal's b). 13. Transverse thoracic diameter at level of axillae, i. e., at 4th costal cartilage. 14. Dorso-ventral thoracic diameter at level of axillae, i. e., at 4th costal cartilage. 15. Dorso-ventral thoracic diameter over nipples. 16. Thoracic circumference at level of axillae on inspiration. 17. Thoracic circumference at level of axillae on expiration. 18. Thoracic circumference over nipples. 19. Suprasternal nipple line, Right, Left. 20. Transverse straight line between nipples. 21. Length of vertical breast contour. 22. Vertical distance of nipple parallel from parallel of substernal notch.

Group III: Abdominal Dimensions. See Notes Nos. 2-14.

23. Smallest circumference of waist. 24. Abdominal circumference at iliac level. 25. Dorso-ventral abdominal diameter at iliac level. 26. Transverse abdominal diameter at iliac level. 27. Transverse pelvic line at level of symphysis pubis = (Friedenthal's d).

Group IV: Appendicular Dimensions. See Notes Nos. 2-14.

28. Circumference of thigh at level of crotch, Right, Left. 29. Circumference of arm at level of axillae, Right, Left. 30. Right upper extremity—upper arm, forearm, hand extended, (inc. carpus). 31. Right hand—middle metacarpal, phalanges I, II, III. 32. Right little finger—phalanges I, II, III. 33. Left upper extremity—upper arm, forearm, hand extended, (inc. carpus). 34. Left hand—middle metacarpal phalanges I, II, III. 35. Left little finger—phalanges I, II, III.

Group V: Cephalo-Spinal Dimensions. See Notes Nos. 2-14.

36. Cranial length. 37. Cranial width. 38. Cranial height. 39. Cranial circumference. 40. Frontal arch. 41. Frontal radius. 42. Height of head.

DIVISION B. GENERAL ANATOMICO-PHYSIOLOGICAL AND NEUROLOGICAL
OBSERVATIONS AND TESTS.

Group VI: Skeleton, Figure, Posture, Locomotion. See Note No. 15.

51. Proportions, observable. 52. Skeleton, general and extremities—bones, large, small, medium, normal. Extremities, normal or curved. 53. Deformities. Description of anatomical condition. 54. Cephalic skeleton. Size, conformation, sutures. 55. Thoracic skeleton. Pathological types. 56. Abdominal protrusion. 57. Peculiarities in posture. 58. Gait. 59. Locomotion to point, blindfolded. 60. Ability to heel and toe a line.

Group VII: Skin, Hair, Nails, Sweat Glands, Fat. See Note No. 15.

61. Color and surface of skin. 62. Color and texture of hair, comparative of scalp, axillae, genital region, other surfaces. 63. Growth and distribution of hair. Abdominal hair line. 64. Nails. 65. Sweat glands. 66. Fat, quantity and distribution.

Group VIII: Mouth, Nose, Throat, Ears, Eyes. See Note No. 15.

67. Conformation, especially of arch. 68. Teeth. 69. Tongue and its glands. 70. Tonsils. 71. Pharynx and adenoids. 72. Ears. 73. Eyes.

Group IX: Genitals and Superficial Glands, Pelves. See Note No. 15.

74. Developmental stage, size, glandular conditions. 75. Deformities or abnormalities in secondary sexual organs. 76. Anatomical internal genito-urinary conditions. 77. Genito-urinary functional activity. 78. Anatomical rectal conditions, and rectal functional activity. 79. Inguinal. 80. Mamillary-axillary. 81. Cervical. 82. Thyroid.

Group X: Internal Organs—Cardio-vascular, Pulmonary, Abdominal. See Note No. 15.

83. Vaso-motor conditions as observed in the skin. 84. Position of apex beat. 85. Character of sounds by stethoscope. 86. Pulse: Rate difference lying and standing, regularity or intermittence, force. 87. Blood pressure, lying, standing. 88. Respiration: Type, number. 89. Spirometer. 90. Abdominal conditions.

Group XI: Neuro-Muscular Organs and Reflexes. See Note No. 16.

91. Automatism. 92. Dynamometer. 93. Tremor of spread fingers. 94. Pupillary and conjunctival. 95. Knee jerk. 96. Plantar. 97. Babinski. 98. Ankle. 99. Epigastric. 100. Abdominal. 101. Sensitivity to needle. 102. Sensory defects. 103. Speech defects.

Group XII: Mental, Especially Intelligence Tests. See Notes Nos. 2-14.

Group XIII. Blood: Cytology, Immunology, Biochemistry.

110. Absolute blood count. 111. Differential blood count. 112. Hemoglobin. 113. Coagulation time. 114. Wassermann reaction. 115. Tuberculin reaction.

Group XIV. Nutrition and Metabolism: Urine, Nitrogen, Calcium, Phosphorus, Chlorine, Sugar, Respiratory, Exchanges.

120. Quantity, color, specific gravity of 24-hour urine. 121. Quantitative acidity of 24-hour specimen. 122. Albumen reaction of 24-hour specimen. 123. Sugar reaction of 24-hour specimen. 124. Quantitative calcium oxide of 24-hour specimen. 125. Quantitative phosphoric acid of 24-hour specimen. 126. Quantitative chlorine of 24-hour specimen. 127. Urinary sediment, inorganic, of 24-hour specimen. 128. Urinary sediment, organic, of 24-hour specimen.

NOTES ON TECHNIC AND REFERENCES.

It is evident that the qualifications of training and experience that *can* be applied in the above plan of examination and study ranges from that of the instructed laboratory assistant to that of the medically skilled and experienced professional person. An intelligent and educated person whose interests lie in this direction can learn to do nearly all of it in a satisfactory way. This statement applies to the ascertainment of facts but by no means to their interpretation and utilization. The scientific relations of many of these topics and their pathological significance must be left for illustration and discussion in the reports of cases so studied and which will be subsequently published. It would obviously require a booklet rather than a paper to enter into the details of the above technic. We shall therefore refer to the following sources of information which have been selected with special reference to the assistance they can give in the use of the above outline. This list does not constitute a bibliography of the topics involved but it is a sufficiently comprehensive guide to the necessary information regarding methods of procedure, instruments and apparatus required, as well as a further index to the literature.

On the general topic of growth and development:

(1) Friedenthal, Hans: *Allgemeine und spezielle Physiologie des Menschenwachstums*. Berlin, Springer, 1914. Pp. X + 161.

Contains a good bibliography on human growth.

On Groups I to V, Anthropometry, and Group XII, Mental, Especially Intelligence Tests:

(2) Report of Anthropometric Investigations, British Isles. Reprinted with additional illustrations by permission of the Council from the Report of the British Association (Dublin Meeting) 1908. Pp. 351. ff.

(3) Montessori, Maria: *Pedagogical Anthropology*. New York, Fred. A. Stokes Co., 1913. Pp. XI + 508.

(4) Hastings, Wm. W.: *Manual for Physical Measurements*. 1902. Pp. XVIII + 112.

Contains anthropometric tables for each age and sex from 5-25 years, also vitality coefficients.

(5) Pyle, William H.: *The Examination of School Children. A Manual of Directions and Forms*. New York, Macmillan Co., 1913. Pp. 67.

(6) Yerkes, Robert M.; Bridges, James W.; Hardwick, Rose S.: *A Point Scale for Measuring Mental Ability*. Baltimore, Warwick & York, Inc., 1915. Pp. 218.

(7) Haines, Thomas H.: *Point Scale Ratings of Delinquent Boys and Girls*. From *Juvenile Research Bureau*, Columbus, Ohio. *Psychological Review*. Vol. 22, No. 2, pp. 104-109. March, 1915.

(8) Haines, Thomas H.: *Diagnostic Values of Some Performance Tests*. Bureau of Juvenile Research, Columbus, Ohio. *Psychological Review*, Vol. 22, No. 4, pp. 299-305. July, 1915.

(9) Goddard, Henry H.: *Standard Method for Giving the Binet Test*. Reprint from *The Training School Bulletin*, Vol. 10, No. 2, pp. 23-30. April, 1913.

(10) Healy, William: *The Individual Delinquent. A text-book of diagnosis and prognosis for all concerned in understanding offenders*. Boston, Sittle, 1915. Pp. XVI + 830.

(11) Healy, William, and Fernald, G. M.: *Tests for practical mental classification*. Lancaster, Penn., Review Pub. Co., 1911. Pp. VII + 53.

(12) Whipple, Guy M.: *Manual of Mental and Physical Tests. Part I: Simpler Processes. Part II: Complex Processes*. Baltimore, Warwick & York, Inc., 1915.

Part II, p. 324, presents a very well selected list of references on *Serial Graded Tests for Developmental Diagnosis*, by means of which the reader can orient himself on this topic.

(13) Thorndike, Edward L.: *An Introduction to the Theory of Mental and So-*

cial Measurements. 2nd ed. Published by Teachers College, Columbia University, New York, 1913. Pp. XI + 277.

(14) Stern, W.: *The Psychological Methods of Testing Intelligence*. Translated by Guy M. Whipple. Baltimore, Warwick & York, Inc., 1915.

On Groups VI to X:

(15) Eisendrath, Daniel L.: *A Text-Book of Clinical Anatomy*. 2nd ed. Philadelphia, W. B. Saunders Co., 1907. Pp. 535.

On Group XI:

(16) Hunt, Robert L. *Diagnostic Symptoms in Nervous Diseases*. Philadelphia, W. B. Saunders Co., 1914. Pp. 229.

On Groups XIII and XIV:

(17) The preceding parts of the above Outline, i. e., Groups I to XII pertain approximately to subheads A to E of the General Plan. The material of Groups XIII and XIV belongs more properly under Subhead F, which is essentially a research topic. Here each competent worker will want to make his own selection or invention of method adapted to his special purpose. For routine purposes the standard works on diagnostic technic will suffice.

(18) For such measurements as are taken standing, a certain uniform normal standing position must be adopted, e. g., heels in contact, body erect as is natural to the individual and not a straight pose. In measuring certain dimensions the distinction should be observed between distances measured on or across the body surfaces as contours or profiles and those of the nature of diameters and to be measured as straight lines between designated points or their projection in space.

(19) The Arm Span, (No. 4) should be taken as the distance measured on a straight horizontal line between the extreme points of the two middle fingers when the arms are extended at right angles to the trunk. Note that it is not correctly or accurately measured across the surface of the chest.

(20) The Total Basal Profile Bf, (No. 9) is measured on the anterior surface of the trunk from the suprasternal notch to the lower surface of the symphysis pubis. It is not commonly a straight line. Normal standing position.

(21) Section Bf1 of the basal profile, (No. 10) extends from suprasternal notch to the substernal notch, Bf2 from latter to navel, Bf3 from navel to lower margin of symphysis pubis. Normal standing position.

(22) Drawing of basal profile, (No. 10a) should be made to scale for the three sections and should correctly show the natural curvatures or angles as seen from a side view taken in normal standing position.

(23) The Basal Line B, (No. 11) should be taken as the distance measured on a straight vertical line from the level of the suprasternal notch to the level of the lower margin of the symphysis pubis. It is not a contour or profile line. Normal standing position.

(24) The suprasternal nipple line is measured in a straight line from the suprasternal notch to the right and left nipples respectively, regardless of sex or in any case of what the position of the nipples may be.

SUMMARY.

(1) The pathology of mental defectives including especially the feeble-minded is based on the principle that their condition, both physio-pathological and psycho-pathological is due to physical abnormalities of growth and development which affect not only the neural but also other systems of organs by reason of intimate hormonal or neural correlation or both. In the majority of cases these begin with the parentage, are continued and magnified during the period of gestation, and are deeply impressed in the physical constitution of the individual so that abnormal growth and development characterizes such individual not only during gestation but continuously after birth. In acquired defect the incidence of the deviation from the normal occurs at a later period in the cycle of development.

(2) An outline of a plan, based on the principle of growth-development, for the examination, diagnosis and further study of mental defectives, has been presented. This outline places emphasis on the physio-pathological factors as well as on the psycho-pathological and the facts thus ascertained constitute the logical basis for further intensive study of the defective individual by physiological and biochemical methods.

—Training School, Vineland, N. J.

LABORATORY METHODS

A Method of Sterilizing Sputum Before Examination*

BY DON M. GRISWOLD, M.D., DETROIT, MICH.

THE question of safety for persons handling tuberculous sputum in routine examination is one that from time to time receives some attention.

Between the extremes of wearing rubber gloves and a surgical operating gown and an utter indifference for personal hygiene and safety lies some rational procedure.

Many laboratories, both municipal and private, refuse to accept specimens in leaking containers. The safety of those engaged in this work demands this protection. Some laboratories send out sputum bottles containing a half ounce of a weak carbolic solution. This inhibits the reproduction of the accompanying organisms and leaves the field less confusing when searching for the tubercle bacilli.

The danger, however, is often on the outside of the bottle or carton, as well as on the inside.

The skillful manipulator will seldom become contaminated with the contents of the bottle, but the bottle or carton must always be handled. Thus it would seem important that if an effort is to be made to protect laboratory workers the whole outfit should be sterilized.

It has been the practice of the laboratory of the Detroit Board of Health to autoclave all sputum bottles after the contents have been examined, then send them to the dump. It was desired to study the effect of this treatment upon the sputum.

First it was found that almost regardless of the physical characteristics of the sputum before autoclaving, after this treatment the sputum was divided into two distinct layers. One of these is thin and watery and probably represents the saliva which was in the bottle. The other resembles soft boiled white of egg. It is sometimes discolored and may be of a greenish or brownish tinge. This is probably coagulated albumen from the sputum. As this is coagulated and falls to the bottom it engulfs and drags with it many of the bacteria contained in the sputum.

When this coagulated material is smeared it will be found that a comparatively even smear can be made very quickly. It is about the consistency of butter.

In a series of two hundred sputa examined first in the usual way, and then after autoclaving, the following results were obtained:

1. All sputa found positive in the usual way were found positive after autoclaving.
2. Among the sputa found negative (150) in the usual way, three showed tubercle bacilli after autoclaving.

The staining characteristic of the tubercle bacillus is not altered so far as we have found. The organism retains the carbolfuchsin in the presence of

*From the Laboratory of the Detroit Board of Health.

15 per cent nitric acid or 3 per cent hydrochloric acid in alcohol. In fact it appears that the organism takes the stain better, but this may be due to the fact that the smear is of much more uniform thickness and a better view is obtained of the stained organism.

The finding of three positives among one hundred and fifty negatives does not indicate that this method is any more accurate than the usual method. It is entirely possible that the careful search of a second slide would have given an equally high result. Studies to show the relative accuracy of the Elliman-Erlandsen, Antiformin, and the Kinyoun¹ methods of digestion of the sputum and concentration of the tubercle bacilli, show a higher percentage of positive results among sputa negative by the usual method.

Agar slants were made of the supernated liquid and the coagulated material of all this series. No such tube ever showed any growth. Guinea-pigs were inoculated with those that showed the presence of acid-fast bacilli. No pigs died or showed the presence of tubercles at autopsy.

The thermal death point of the tubercle bacillus is between 55° C. and 65° C. for ten minutes.² In autoclaving sputum bottles, the temperature is raised to 120° C. with twenty pounds pressure for twenty minutes.

The advantages of the method are:

1. No special apparatus is necessary.
2. The outside of the bottle and carton are sterile.
3. Nothing is added to the sputum.
4. Small amount of time consumed.
5. Smears can be made more rapidly as no effort is necessary to select a particular piece of sputum.
6. The contents of the bottle are sterile.

Many of these specimens have been counterstained with picric acid.² When using the picric acid counterstain, the smears should be fixed and stained with carbolfuchsin in the usual way. An electric hot plate covered with asbestos board serves very well for the heating. The following solutions should be kept in wide-mouth bottles so that the smeared part of the slide can be immersed.

The smear should be immersed in one bottle after the other without washing.

Bottle No. 1: Saturated aqueous solution, picric acid and absolute alcohol; equal parts; about three seconds.

Bottle No. 2: Alcohol 60%; about three seconds.

Bottle No. 3: Nitric acid 15%; until properly decolorized.

Bottle No. 4: Alcohol 60%; about three seconds.

Bottle No. 5: Saturated alcoholic solution of picric acid; until a distinct yellow color is seen.

The contrast is fully as good as with the blue counterstain when the eye becomes accustomed to the change.

The advantage of the method lays in the fact that a smear five times the usual thickness can be examined because the picric acid is not an opaque stain. It is somewhat confusing at first to focus up and down through five superimposed fields, but the labor entailed is much less than making separate smears.

¹Amer. Jour. Infec. Dis., Jan., 1914, p. 159.

²Hewlett: Manual of Bacteriology, Mosby Co., St. Louis, 1914.

Slide Holder and Protector for the G. S. & E. Warming Stage*

BY C. R. ECKLER, INDIANAPOLIS, IND.

ONE of the most widely useful accessories for the microscope in the field of biology is the warming stage and incubator. For observation of bacteria and yeasts in the hanging drop, for the study of malarial parasites, trypanosomas, amoebæ, or other motile organisms, for the carrying out of Widal tests, etc., such an instrument is not only of inestimable value but is almost indispensable.

A very convenient electrical warming stage and incubator is found in the No. 8, made by the Chicago Surgical and Electrical Company. As a stage incubator this instrument seems quite complete (Fig. 1), and for a description of it, quotation may be made from a booklet supplied by the Arthur H. Thomas Company, Philadelphia.

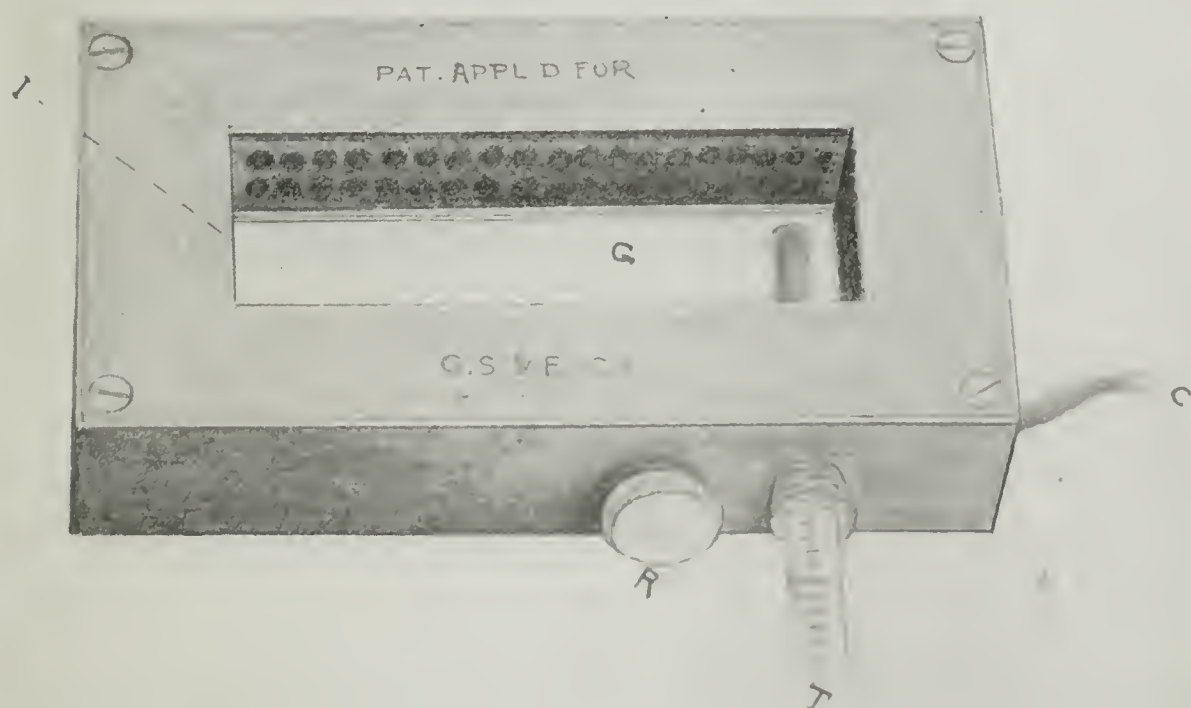


Fig. 1.—Warming stage and incubator. Mica cover for incubating chamber not shown. C, Cord from resistance lamp; T, Thermometer; R, Thumb-screw for regulating temperature; I, Incubating chamber; G, Glass slide at bottom of incubating chamber.

“The outside dimensions are $3\frac{1}{2}$ inch. \times 2 inch. \times $\frac{1}{2}$ inch in thickness. The incubating chamber measures 3 inch. \times 1 inch \times $\frac{1}{2}$ inch in depth and has a removable glass bottom consisting of a regular 3×1 slide. The specimen to be examined is placed on this slide. The condenser may thus be brought up close to the glass, same as under ordinary conditions, permitting the use of the oil immersion lens.

“To cover top of incubator we furnish a piece of clear mica having a hole cut in center through which the lens is passed when making adjustment for

*From the Department of Experimental Medicine, Eli Lilly & Company.

the bottom slide. A rubber washer slipped over the lens is provided for sealing the opening between the lens and mica.

"By this arrangement a perfect incubating chamber is obtained, where bacteria may be observed for any length of time.

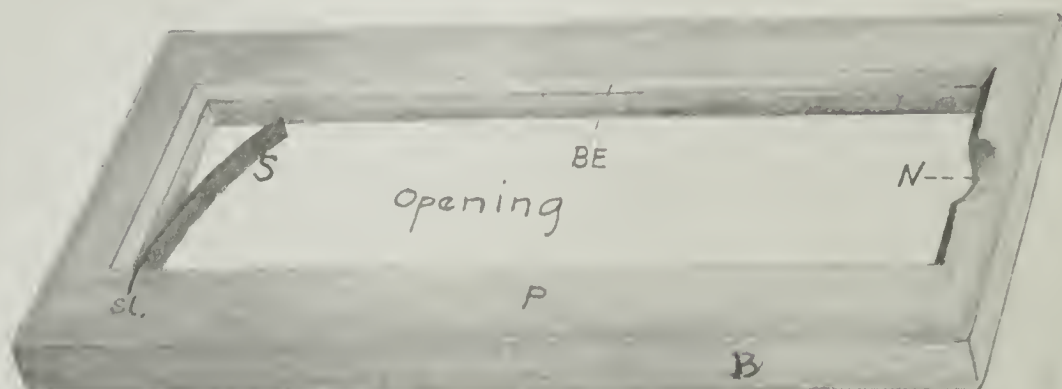


Fig. 2.—The slide holder and protector. *S*, Clock spring; *N*, Notch to admit finger nail; *BE*, Beveled edge of plate; *B*, Band 5 mm. wide about plate; *P*, Plate 4 mm. thick.

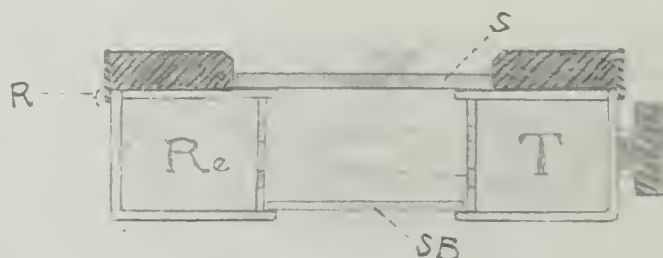


Fig. 3.—Cross-section of incubator and slide holder. *T*, Thermostat element; *Re*, Resistance coil; *S*, Regular microscopic slide; *R*, Rim which fits over incubator; *SB*, Slide at bottom of incubating chamber.

"The incubator is complete in every detail with heating element, thermometer and adjustable electro-thermostat, which automatically maintains a constant temperature inside the incubating chamber, as long as the electric current is on. This device can be adjusted for any temperature between 80° and 110° Fahrenheit and is very accurate.

"To operate, simply screw the current tap with its lamp into a 110 volt electric lamp socket and turn on the current."

This description appears to be correct except for the first paragraph in reference to the size of the incubating chamber (I. Fig. 1), which measures approximately $2\frac{3}{8}$ inches \times $\frac{3}{4}$ inch \times $\frac{1}{2}$ inch, instead of 3 inches \times 1 inch \times $\frac{1}{2}$ inch, and carries a slide (G, Fig. 1) at the bottom of the incubating chamber measuring approximately $2\frac{3}{8}$ inches \times $\frac{3}{4}$ inch, instead of 3 inches \times 1 inch.

This instrument may be used as well for a warming stage, and perhaps in this way, offers its greatest field of usefulness. For use in this manner it seems quite complete with one exception. Dr. A. L. Walters, using one of these instruments in the study of the endamoeba of pyorrhea, suggested the need of a

slide holder and protector, there being no device on the instrument to hold the slide firmly in position during the extended observation of the motile forms, or to protect the slide from cross currents of air, or to seal any small spaces between the edges of the slide and the top of the incubator. Such spaces were found, owing to the fact that the top of the incubator was not perfectly level at all points. To fill these needs, the attachment here described, was constructed.



Fig. 4.—Showing instrument in position with slide inserted.

This attachment is essentially a cover for the top of the incubator (Fig. 2), made of a plate of brass 4 m.m. thick (P), which has about its edge a band of 22 gauge brass 5 m.m. wide (B) thus affording a rim at the lower edge of the plate 1 m.m. in depth (R, Fig. 3). This rim fits snugly down over the top of the incubator, gentle pressure being required to seat the plate in position, where it is held by friction. An opening (Fig. 2) is cut through the plate large enough

to admit an ordinary microscopic slide, which, when in place, rests on the top of the incubator (S, Fig. 3), closing the upper opening of the incubating chamber. The edges of the plate about this opening are beveled (BE, Fig. 2) so as not to hinder the turning of the objectives. At one end, the opposite end from which the slide is inserted, a curved piece of clock spring (S, Fig. 2) is fastened by soldering in a small slit (Sl.) cut with a thread saw. This spring holds the slide firmly in place during the examination of any object, and in removing the slide, after the end opposite the spring has been released by raising with the finger nail, this spring forces the slide out of the opening to a point where it may be easily picked up with the fingers. The instrument is nickel plated.

The chief advantage of the device is that it holds the slide firmly in position, enabling the operator to observe a small object, such as a single amoeba, as long as desired without danger of the slide being moved about accidentally. Forming a sort of shallow well for the slide, it seals any little spaces between the edges of the slide and the top of the incubator, and protects the slide from cross currents of air. The added weight to the incubator is useful when operated on a plain microscope stage where there are no clips to hold the instrument. The thermal capacity of the attachment aids to some extent in maintaining a uniform temperature.

The attention of laboratory workers is called to this little device through the belief that it is a valuable addition to the G. S. & E. No. 8 Incubator, when used as a warming stage, and that the advantages afforded by it would be appreciated by any one who uses one of these warming stages. It is so simple in construction, and so easily made, that anyone possessing only slight ability as a craftsman may make one for himself with the aid of a few common tools.

Hydrogen-ion Acidity*

By THOMAS H. KELLY, M.D., CINCINNATI, OHIO.

MANY attempts have been made of late years, to evolve methods by which the actual amount of free hydrogen-ions in body fluids might be determined, for the use of laboratory workers and clinicians. In these days of discussion concerning the role of colloids and their changes in hydration capacity in health and disease, an accurate knowledge of variation in hydrogen-ion content of body fluids and tissues is very necessary to a proper understanding and interpretation of many disease processes.

Of all the methods which have been used, the gas chain electro-meter method is the most nearly accurate, but its use in a clinical laboratory is practically barred because of the delicate apparatus and special training in physico-chemical methods necessary to its employment. A great many other methods are likewise too complicated to become matters of routine in clinical laboratories. Perhaps of all the methods, those of S. L. P. Sørensen and of L. J. Hender-

*From the Laboratory of the Cincinnati General Hospital.

son are among the simplest and lend themselves best to routine use, but even these are rather complicated.

In these methods standard mixtures of phosphates or acetates are made, in series containing known quantities of hydrogen-ions, and indicators are added. The fluid to be tested is properly diluted and the same indicators added, and it is then compared with the colors of the standard mixtures. This method has been very valuable and has been recently applied to the determination of hydrogen-ion acidity of the blood, to be referred to shortly.

Martin H. Fischer¹ has developed a method for determining hydrogen-ion acidity of the urine within limits of accuracy that are sufficient for clinical purposes by the use of a series of indicators graded so that their color changes take place at definite hydrogen-ion concentrations. The series which he has found to be the most useful is as follows:

INDICATOR.	CONCENTRATION OF H—ION AT COLOR CHANGE.	COLOR.	
		ACID.	ALKALINE.
Methyl Orange 1-2% aqueous sol.	10^{-4}	Pink	Orange
Para-nitro-phenol 2% alcoholic sol.	10^{-5}	Colorless	Greenish-yellow
Sives red- ² 2% aqueous sol.	10^{-5} to 6	Red	Canary yellow
Methyl red 0. 2% alcoholic sol.	10^{-6}	Magenta	Canary yellow
Rosolic acid 1-2% in 50% alcohol	10^{-7}	Orange	Magenta
Phenolphthalein 1% alcoholic sol.	10^{-9}	Colorless	Bluish-red
Thymolphthalein 1-2% alcoholic sol.	10^{-11}	Colorless	Blue

⁻¹Hydrochloride of para-mono-methyl-amino-azobenzine-ortho carbonic acid.

10 c.c. of urine are placed in a clean porcelain evaporating dish which is rinsed with distilled water or the urine to be tested. The urine should be as fresh as possible. By trying two drops of each indicator on successive samples of the urine to be tested an indicator is finally found to which the urine is neutral, or is acid to one indicator in the series and alkaline to the next above it. Therefore the urine has a hydrogen-ion concentration equal to that represented by the turning point of the indicator in the first case, or lies between the turning points of the two indicators in the second. In this way a fairly accurate approximation of the hydrogen-ion concentration may be obtained.

Recently R. L. Levy, L. G. Rowntree and W. McK. Marriott² have published a method for the determination of the hydrogen-ion concentration of the blood. In this method they use a standard series of tubes containing mixtures³ of 1-15 mol. solutions of primary potassium phosphate (KH_2PO_4) and second-

¹Fischer, Martin H.: *Oedema and Nephritis*, John Wiley & Sons, New York, 1915.

²Levy, R. L.; Rowntree, L. G.; Marriott, W. McK.: *Arch. Int. Medicine*, 1915, xvi, No. 3, p. 389.

³Sørensen: *Biochem. Ztschr.* 1909, xxii, 352.

ary sodium phosphate ($\text{Na}_2\text{PHO}_4\cdot 2\text{H}_2\text{O}$) with five drops of a 0.01 per cent solution of phenolsulphonephthalein added to each tube. These mixtures are made so that each tube has a known hydrogen-ion concentration.

The determinations can be carried out on either serum, plasma, whole or defibrinated blood, and must be done in a room containing no fumes of ammonia or acids.

The blood or serum must first be dialyzed in a thin celloidin sac against an 0.8 per cent salt solution which has been tested for its neutrality. The sac permits the passage of salts but not of coloring matter or proteins.

One to three c.c. of clear serum or blood is run into the celloidin sac which has been tested for leaks and washed inside and out with salt solution. The sac is lowered into a test tube (100 by 10 m.m.—inside measurements) containing 3 c.c. salt solution, so that the salt solution is as high on the outside of the sac as the level of the blood or serum on the inside. Five to ten minutes are allowed for dialysis and then the sac is removed. Five drops of the indicator are thoroughly mixed with the dialysate and it is immediately compared with the standard series and readings made.

For the reading a good light and a white background are necessities, and readings must be made immediately. The tube most nearly approaching the color of the one to be tested is selected, and also the tube on each side. These are then examined carefully against a white background.

The authors have thoroughly tested this method and the variations in repeated determinations are surprisingly small, serum from normal persons varying between $\text{H}—10^{-7.6}$ to -7.8 . Variations toward the acid side were observed only in those cases which clinically and from the laboratory findings showed evidence of an acidosis.

Thus, for the clinical study of variations of hydrogen-ion concentration in health and disease, we have two methods which should prove valuable in the hands of workers in these fields.

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EDITORIALS

New Methods for the Investigation of Breathlessness and Acidosis

MUCH has been written in recent years concerning the supposed existence of conditions in which the reaction of the blood becomes less alkaline, that is, tends to become more acid. For the detection of this acidosis, as it is called, the most sensitive of physico-chemical methods have been adapted, and yet it has been impossible in many cases where we have every reason to expect high degrees of acidosis (as in diabetic coma) to obtain very satisfactory evidences of its presence. On the other hand, even slight disturbances in the reaction of the blood cause very distinct changes in the activity of the respiratory center, conditions associated with acidity making it more active (e. g., the breathlessness of muscular exercise, the injection of acid into animals) and conditions associated with alkalinity depressing it (the apnea produced by injecting alkali). From these observations we must conclude that the respiratory center is more sensitive to change in the reaction of the blood than the most refined physico-chemical methods.

It is obviously, however, most important for the successful investigation of the clinical conditions that are associated with some disturbance in the respiratory function, such as dyspnea and cyanosis, that we should possess a reliable method by which we may detect acidity of the blood and observe its behavior under various forms of treatment. If possible, too, we must find out the nature of the acid which is responsible for the change in blood reaction.

Thanks to the combined efforts of a group of clinical and laboratory work-

ers in London (Lewis, Barcroft, Ryffel, etc.), a great step has lately been taken in supplying us with new and apparently reliable methods. We do not propose at the present time to go into the details of this method; we defer this for a future issue of the Journal, when we hope to be able to offer some modifications which our experience with it has suggested. For the present, however, a brief description of the principle upon which the method depends is advisable, in order that we may appreciate the significance of the work which has already been done, and follow the investigations now in progress which we hope to report on from time to time.

When blood is shaken in atmospheres containing variable, but known, quantities (or pressures) of oxygen, the degree to which the hemoglobin takes up the oxygen at each pressure of the gas depends very largely on the acidity (i. e., the H-ion concentration) of the blood.¹ If the results be plotted, with the values along the ordinates representing the relative saturation of the blood with oxygen, and those along the abscissæ, the partial pressure of oxygen at which the blood was exposed, a curve, called the dissociation curve of hemoglobin or blood, is obtained. This curve has a definite shape and position in the case of normal blood. If some acid be added to the blood, the curve will be found to flatten out in proportion to the amount of acid added. Indeed, the acidity of a sample of blood can be found by comparison of its dissociation curve with that of normal blood to which known quantities of acid have been added. When the curves correspond, the bloods contain the same amounts of acid.

For practical clinical purposes, however, it would obviously be too time-consuming if, in order to plot the curve, we were compelled to employ the above described method of shaking several samples of blood at different oxygen pressures and then analyzing to see how much oxygen had been taken up at each pressure. But this is not necessary, for, thanks to the work of L. V. Hill, it is possible to find all positions of the curve for a given sample of blood by using a *constant*, K , which can be determined by finding the saturation of the blood with oxygen at *one* pressure of this gas. This constant was found by the application of mathematical principles to data furnished by a large series of observations. For determining the reaction of the blood, then, we do not require to actually plot the curve; the constant itself is sufficient. When this is low, for example, it indicates that at a given pressure of oxygen, the blood takes up an abnormally low percentage of its total possible load of oxygen, indicating that the acidity of the blood is greater than normal, and when the constant is high, for the same reason, the acidity of the blood must be low.²

When the blood is within the vessels its acidity is due to the presence in it of two types of acid, volatile and fixed. The volatile acid is the carbon dioxide in solution in the plasma. The fixed acids are the acid salts, lactic acid, and in some cases other acids, such as butyric. On account of the readiness with which carbon dioxide escapes from the blood after its withdrawal, it is plain that in determining the dissociation curve, or constant, we will be incurring an error on account of the loss of carbon dioxide. We will, in other words,

¹It is also influenced by salts, but this factor may for the present be disregarded.

²Barcroft suggests the following terms to express these conditions: (1) meionectic—where K is low and the acidity therefore great; (2) pleonectic—where K is high and the acidity small; (3) mesectic—when K is normal.

find the blood to be less acid than it should be. In order to find the true degree of acidity we must shake the blood in an atmosphere containing the same pressure of carbon dioxide as that which exists in the patient's blood plasma.

To make the method of value, therefore, for measuring the actual H-ion concentration of the blood, we must know the carbon dioxide tension. This is comparatively an easy thing to determine, for, thanks to the work of Haldane and his pupils, we know that the percentage of carbon dioxide in the alveolar air is directly proportional to the tension of this gas in the blood plasma. All we have to do, therefore, is to take a sample of alveolar air and analyze it for its percentage amount of carbon dioxide, and then to add this amount of carbon dioxide to the atmosphere with which the blood is shaken in determining the dissociation constant.

We have described the way in which the method may be used for determining the actual H-ion concentration, but now we must consider its application in detecting *the exact nature of the acid* which may be responsible for hyperacidity. This is done by an application of the same principle and may perhaps be best illustrated by taking a concrete example. Let us suppose that the dissociation constant shows us a given blood, shaken in the absence of carbon dioxide, to be hyperacid (i. e., K low). The question is, of what nature is the acid that is present in excess? We have seen that it may be either volatile or fixed acid. If volatile acid is the cause, then we should expect, from Haldane's principle, that an excessive percentage of carbon dioxide will exist in the alveolar air. Such will actually be found to be the case during the earlier stages of muscular exercise, but in pathological conditions (for example, the so-called uremias) it will usually be found that there is no evidence of an increased amount of carbon dioxide in the alveolar air, indeed, it is usually lower, so that the hyperacidity of blood must be due to fixed acid.

Or let us take the case of diabetic coma. Shaken in an atmosphere that is free of carbon dioxide, the blood will be found to be hyperacid, but if the alveolar CO_2 content is determined, it will be found subnormal; often markedly so. The conclusion is that the hyperacidity of the blood in diabetic coma is not due to an excess of carbon dioxide, but to an excess of fixed acid, which has displaced much of the carbon dioxide.

To illustrate the value of the above method, a few of the results which have already been obtained by Lewis, Barcroft, etc., may be of value. In the dyspnea which occurs in mitral stenosis of young rheumatic girls, the blood, freed from carbon dioxide, has a normal reaction, but the alveolar air contains an excess of carbon dioxide. The dyspnea in these cases is therefore due to a hyperacidity caused by a mechanical defect in the ventilation of the lungs—a defect which interferes with the proper excretion of carbon dioxide.

In another group of cases which would commonly be diagnosed as uremic, and in which dyspnea rather than cyanosis is the pronounced symptom, the blood, freed from carbon dioxide, is found to have a high acidity (K of low value). On the examination of the alveolar air, however, a low tension of carbon dioxide is found present. Evidently, then, the acidity is due to the excessive presence of non-volatile acids.

Another instance of the use of the method is in diabetic coma. As already

mentioned, in this case the carbon dioxide-freed blood has a very high acidity, and yet the alveolar CO_2 tension is very low. And, lastly, in cases of mountain sickness the blood samples show high acidity, but the alveolar air, a low carbon dioxide tension.

Before this work can be considered as finally completed, we must succeed in showing that there is really an excessive amount of fixed acid present in the blood. We must isolate this acid and see what it is. Although this part of the problem is being vigorously attacked, it has not as yet been possible to succeed in showing that all of these cases having high fixed acidity really contain an excessive amount of lactic acid in the blood. Sufficient evidence has, however, been accumulated to show that the above inferences are probably correct.

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—J. J. R. M.

The Welch Bacillus and Disease

THE recent frequent reports of so-called "gas bacillus" infections among the soldiers in France has lent a peculiarly timely interest to the monograph of the Rockefeller Institute on "Studies in *Bacillus Welchii*, with Special Reference to Classification and to Its Relation to Diarrhea," by Dr. J. P. Simonds. There has been endless confusion in the classification of the so-called aerogenes capsulatus organisms, a group which has been known in different countries by a large variety of names. Its first description is probably that by Welch and Nuttall in 1892, who gave it the name *B. aerogenes capsulatus*, by which name, or briefly *Welch bacillus*, it is most commonly designated by American bacteriologists. Similar or identical organisms are the *B. phlegmonis emphysematosae* of Fraenkel, the *B. enteritidis sporogenes* of Klein, the *B. perfringens* of Veillon and Zuber, and the *Granulobacillus saccharobutyricus liquefaciens immobilis* of Schattenfroh and Grassberger, and a number of others less prominent in the literature. The task of classifying these organisms is one of great technical difficulty, and, as in so many other groups of bacteria originally described as a single species, we are now learning that instead of finding complete identity of individual isolations, one with another, we can merely draw a circle about a correlated group, recognizing close relationship morphologically, culturally, and in relation to infections of man and animals. After a very careful study of many different strains, Simonds comes to the conclusion, that the Welch bacillus, so-called, is roughly identical with those mentioned, including also the bacilli once associated by Achalmé with rheumatic infections. The term *B. Welchii*, he concludes, does not represent a fixed species but a closely related group of bacteria not yet fully classified even by his own extensive studies.

It is hard to determine in the case of organisms of such varied activities exactly what can be regarded as a cardinal characteristic important for classify-

ing purposes. The organisms treated of in this group are all large Gram-positive, non-motile bacilli, with rounded ends, rarely occurring in chains, and anaerobic. Spore formation is inconstant and occurs only in an alkaline medium. When fermentable sugars, and consequently free acid, are present, no spore formation takes place. Milk is fermented with the formation of butyric acid. The intravenous injection of animals, especially rabbits, usually produced death with an enormous swelling of the body by the formation of gas, which burns with a pale blue flame. Capsules may be seen when the smears are made directly from animal tissues, but are almost universally missed in organisms taken from culture.

As to minor cultural characteristics, so many variations are observed that it is hardly worth while to summarize them in a brief review of Simond's monograph. It is extremely interesting that, though a pathogenic anaerobe, no one has so far been able to show satisfactorily the production of a true exotoxin, though such a claim has been made several times. Poisonous products that have been obtained from cultures have not conformed with the characteristics of true exotoxins as we recognize them now. Moreover, the reaction of toxin-containing filtrates, as Simonds points out, has been almost always acid, and McCampbell and others have suggested that the toxicity of such cultures is due to the presence of butyric acid. It is interesting in this connection also that Herter, who gave a great deal of attention to the presence of these organisms in the bowel and attributed to them etiological relationship with many intestinal disorders, believed that the diarrhea following intestinal putrefaction was due to a very large extent to the irritation produced by ammonium butyrate, the Welch bacilli of the bowel being responsible for the appearance of the butyric acid.

The search for endotoxins, too, seems to have been unsuccessful, and hemolysins have been irregularly present in cultures studied by various investigators.

Great differences in virulence have been observed in the study of various strains. A good many of the strains seem to have great pathogenicity for the ordinary laboratory animals, especially for guinea-pigs and rabbits. Yet certain non-sporulating forms have shown little or no virulence. However, variation in virulence seems to fluctuate considerably according to the culture medium and the symbiotic conditions under which the organisms have been cultivated. In man, fortunately, there seems to be a relatively powerful resistance against Welch bacillus infections. In fact, many investigators have believed that the Welch bacillus is primarily a saprophyte and requires the presence of dead tissue or physiological injury before it can penetrate and thrive in the human body.

Very interesting is the extensive review of the literature in which this organism or varieties of it have been found associated with human lesions. It has been observed in gas cysts of the brain, in "gangrenous" pneumonia, in appendix abscesses, in puerperal sepsis after abortion, in general septicemia, and in many other conditions. However, in many of these cases it may be quite reasonable to question the primary character of the "gas bacillus" infection, and in others again, the Welch bacillus may be regarded as an ante-mortem invasion, a supposition strengthened by the study of terminal infections made by Flexner, in which such a possibility is plainly demonstrated. Thus, while the Welch bacil-

lus may unquestionably invade the human body and do much injury, and even be probably regarded in many cases as the direct cause of death, its invasion in many of these cases must be regarded as not a primary invasion, but as one associated with and made possible by either traumatic injury or the co-operation of other invasive germs such as streptococci, staphylococci, and others. There may, of course, in addition to these be rare instances in which the Welch bacillus, owing to excessive virulence of the strain or relatively low resistance of the infected individual becomes the primary invader. It is easy to understand under these conditions, why in warfare, severe trauma associated with the carrying of soil and often feces-contaminated fragments of clothing into the wound would form ideal conditions for the production of Welch bacillus infections.

The most interesting chapter in the pathogenicity of the Welch bacillus is its relation to intestinal disease. Simonds confirms by his own work the work of many others which has shown that the Welch bacillus may be regarded as a normal inhabitant of the intestines of man. In nineteen babies under one year of age he found Welch bacilli in the stools of eight. He points out that it has been found even in nursing infants by a number of investigators. His own cases were feeding cases with practically no digestive disease and without pus or blood in the stools in any of them. Fluctuations in the abundance of Welch bacilli in the stools of adults, Simonds attributes very largely to daily variations in diet. He thinks it quite likely that the bacillus may give rise to a fairly definite type of diarrhea. He summarizes the arguments brought out in favor of this, as follows:

1. In cases of this kind spores are found in great numbers in the stools.
2. Patients with diarrhea associated with excessive number of gas bacilli are made worse by feeding them a diet rich in carbohydrates and show improvement when the diet is changed to one of pure protein with or without buttermilk.

The relation of the organism to pernicious anemia, first pointed out by Herter, Simonds dismisses with a rather brief discussion, coming to the conclusion that no positive statement can at present be made concerning the relation of the bacillus to this disease. He isolated four strains from stools of patients with pernicious anemia, one of which caused no hemolysin at all, two others being very slightly hemolytic. Of course, the anemia of pernicious anemia is not necessarily due to absorption of hemolysin and might be regarded as secondary to injuries to the digestive tract and the digestive processes, coincident to the constant presence of large numbers of these organisms in the intestinal contents. However, with Simonds, it seems to us from a reading of the literature that no satisfactory relationship between the organism and the disease has as yet been established.

In spite of the fact that the Welch bacillus does not produce spores in culture media in the presence of fermentable substances or of free acids, nevertheless under the conditions of symbiotic growth in the intestine Simonds shows that sporulation is possible even in the presence of fermentable carbohydrate provided that the symbiotic flora is such that the acidity of the mixture does not rise above three per cent, measured in normal acid. Taking this into consideration Simonds concludes that the number of spores of *B. Welchii* in the

stools is a reasonably accurate index of the number of actively fermenting, disease-producing organisms of this type higher up in the intestine.

Although the subject of Welch bacillus infections is by no means closed by Simond's monograph, it nevertheless goes far to bring order out of chaos, and it is quite plain that no conclusions concerning this group can be attained without the comparative study of a large number of different strains.

—H. Z.

Carbon Dioxide and Blood Pressure

THE mechanism which controls blood pressure has supplied many workers with interest through many series of experiments. The facts relating to blood pressure are chiefly two; i. e., it may be modified by alteration in the peripheral circulation, or by increase in the force of the heart beat.

The physiologic method of modifying the peripheral vascular pressure is one which modifies the condition of contraction of the muscle of the smaller arteries. If they are contracted, the blood pressure rises; if they are dilated, it falls. The cardiac output is conditioned by the state of the heart muscle and by the cardiac intake. The former is governed primarily by the coronary circulation; the latter, by the conditions in the peripheral circulation. When increased work is thrown upon the myocardium, the coronary vessels dilate and so the nutritive conditions in the myocardium are improved. The dilatation of the coronaries is determined by chemical means; i. e., by the metabolic products in the blood, mainly, perhaps, carbon dioxide.¹ There seems to be no doubt that acid products of metabolism are the main conditions underlying vascular dilatation. This would obviously tend to the conception that under conditions of increased metabolism, the blood pressure should fall—which it does provided the CO₂ tension passes a certain stage. Within certain limits, however, increased CO₂ tension results in a rise of blood pressure.

In 1909 Kaya and Clark showed that an increase of CO₂ tension in the blood was followed by increased blood pressure, and that decreases in this tension were followed by proportionate decreases in blood pressure.

Cathcart and Clark later showed that deep anesthesia inhibited this mechanism, and Jerusalem and Starling found that when they used an isolated heart-lung preparation an increase of CO₂ caused slowing of the heart, while at the same time the ventricular output was increased by addition of CO₂ up to 8 per cent of an atmosphere. Itami also found that with 5-10 per cent of CO₂, the blood pressure rose, and von Anrep demonstrated that if the adrenals were removed or isolated, the CO₂ rise of pressure did not occur. Cannon and Hoskins and Czubalski observed that in asphyxia there was increased adrenalin in the blood.

Cathcart and Clark² have recently reported experiments which were planned to discover the mechanism of the process indicated in the above mentioned paragraphs. They found that in the intact animal an increase of CO₂ is followed by an increase of blood pressure. In their experiments they used 12 per cent CO₂. The heart was always slowed. When pure air was substituted for the

¹Markwalder and Starling: Jour. Physiol., 1913 (47) 275. (For other references, cf. Woolley: Jour. Amer. Med. Assoc., 1914 (63) 2279, and Centrallb. f. all. Path., 1913 (26) 217.)

²Jour. Physiol., 1915 (49) 301.

CO₂ the cardiac amplitude rapidly returned to normal and the blood pressure fell gradually. If the animal was decapitated, the cardiac rate and amplitude, and the blood pressure fell. Section of the vagi *alma* did not prevent a rise of pressure.

These experiments make it quite evident that some part is played in the production of increased blood pressure by the higher centers, and that this influence is removed by deep anesthesia and decapitation, but not by section of the vagi. In view of the results of von Anrep, Cathcart and Clark treated animals with nicotin and then administered CO₂. Under these circumstances there was no increase in pressure, which indicated that, as Cannon has said, adrenalin plays a part in the regulation of blood pressure.

From these results one is justified in believing that increased CO₂ content in the blood increases the adrenalin content of the blood and this in time causes vascular constriction and increased blood pressure, associated with slowing of the heart and increased amplitude. The results of Cathcart and Clark indicate that the mechanism may be broken by removing the influence of the sympathics upon the adrenals, or by interference with a central mechanism which, they suggest, is in the medulla. Whatever the mechanism is, small doses of adrenalin given intravenously will still produce their characteristic reaction.

All this is very interesting from the standpoint of organic chemical correlations. As work is done by the tissues of the body, especially the muscles, the acid products of metabolism enter the blood stream and being carried to the respiratory center, stimulate it and so produce an increased respiratory rate. This, in its turn, tends to prevent an over-increase of CO₂ in the blood. At the same time the moderate increase of CO₂ which appears in the blood stream acts, by way of the adrenal mechanism, in producing increased peripheral resistance, increased blood pressure, and slowing of the heart, and thereby increases the ventilation of the tissues, and increases the facilities for washing them out. In a recent report Boothby³ shows the relationship by a series of experiments and the results of these lead him to the conclusion that one factor; i. e., the total acidity of the blood, or the hydrogen-ion concentration in the arterial blood, is the governing cause of both circulatory and respiratory activities.

—P. G. W.

Bismuth Poisoning

THE importance of bismuth in medicine has greatly increased within the last few years. Various manufacturing interests bearing this extended use in mind have now placed on the market a large number of new compounds containing the metal. As a general rule the value of these in medicine depends on their difficult solubility. For while a certain rather limited value attaches to these preparations as antiseptics after they pass into solution either in the tissue fluids or in other menstrua, we are as yet practically entirely ignorant of any special therapeutic value which these compounds may possess over various other similar rather insoluble substances. They are easily accessible and serve

³Amer. Jour. Physiol., 1915 (37) 383.

a number of purposes well and have therefore come more and more into use.

The older uses of the salts of bismuth as dusting powders for ulcers, for gastric catarrh and gastric ulcer and for diarrhea or other inflammatory conditions of the mucosa of the alimentary tract have more recently been greatly overshadowed by the introduction of bismuth salts into x-ray work and by their use in the diagnosis or treatment of chronic abscesses or sinuses. For this latter use the medical profession is mainly indebted to Doctor E. G. Beck, of Chicago, who has opened up a practically new field in modern therapeutics by the introduction of a paste¹ composed essentially of 33 per cent of a bismuth salt (usually the subnitrate, $\text{BiONO}_3 + x\text{H}_2\text{O}$) and 66 per cent of vaseline. Occasionally wax² is used in the paste. Soluble bismuth preparations are of but little use in the medicine for they are very corrosive. Applied locally to ulcerated or abraded surfaces the common bismuth salts such as the subnitrate or subcarbonate [$(\text{BiO})_2\text{CO}_3 + x\text{H}_2\text{O}$], gradually take up a certain portion of the tissue fluids and form a protective pellicle or crust which may expedite the process of healing. A mild antiseptic action and some astringency are also manifested by the metal under these conditions.

Within the last few years a special interest has been developed in this subject by the occurrence of a number of cases of bismuth poisoning. Clinically, these have usually been due either to absorption from the alimentary canal following ingestion of the metal for the purpose of x-ray diagnosis or to absorption from a sinus or abscess injected with Beck's paste.

Experimentally only a limited amount of work has been done on the pharmacological action of the metal. There appear to be a great many points on which further careful experimental work is greatly to be desired. Under most conditions the metal is only a feebly active body and this perhaps accounts for the little consideration which has generally been accorded to it by pharmacologists. The work which has been done, however, serves to give some insight into its pharmacological and toxicological properties. In animals acute poisoning may readily be produced, or the slow absorption of small quantities over some time may lead to chronic intoxication. If a soluble double salt such as the tartrate of sodium and bismuth be injected into an animal, there will be produced an acceleration of the respiration, irregular clonic and tonic convulsions followed by prostration, incoordination and weakness. The heart is first slowed and later becomes irregular and weak. The central nervous system is first stimulated and later paralyzed. This stimulation of the central nervous system, which appears to be most marked in the medulla, is of peculiar interest in a metallic substance. There is a progressive fall of blood pressure as the heart becomes weak and irregular, and the vasomotor centre is more and more depressed.

The chronic form of poisoning appears to resemble the symptoms produced by prolonged absorption in man quite closely. The condition here reminds one especially of a mild form of chronic mercury poisoning with some features resembling chronic lead intoxication. The alimentary tract is first affected and anorexia, nausea and vomiting, diarrhea, increased salivation and soreness of the mouth, tongue and gums come on. A peculiar narrow violet-black line may

¹Beck, E. G.: *Annals of Surgery*, 1914, lix, p. 145.

²Jensen, Theodor: *Muenchener Medizinische Wochenschrift*, 1913, ix, June 3, p. 1202.

appear on the margins of the gums as one of the first symptoms. This is liable to persist for a considerable period. If the case be mild, this may be the only marked symptom present. The urine often contains bismuth and may be of a dark color. If the intoxication proceeds the stomatitis becomes more and more severe. Ulceration of the gums and buccal mucosa ensues and peculiar violet or blackish spots appear over the surface. These may become infected and lead to fever and general systemic disturbances. The swollen gums may bleed and the teeth may become loosened or fall out. White diphtheritic-like membranes have been described as appearing on the gums. Various forms of convulsive manifestations may appear from time to time, and pain and difficulty are experienced in swallowing. Albuminuria is generally present especially if the case is severe.

From the alimentary canal bismuth appears to be absorbed only with difficulty and in very small amounts under ordinary conditions, so that poisoning from this source by bismuth itself is rare. When taken into the stomach or intestine the metal is supposed to form a thin protective covering over the mucosa and thus protect ulcerated or abraded surfaces. From various experimental and clinical observations it appears that absorption of the substance is much more likely to occur if a large area of raw or granulating surface is coated with the metal than if it comes in contact only with the normal mucosa. This apparently also holds true to a certain extent in the injection of sinuses and abscesses, for if these are old chronic lesions and no probing or cutting of the tissues has been carried out so that the paste cannot come into immediate contact with a fresh surface of the tissue, then absorption and poisoning are much less likely to occur. In 1887 Dalché and Villejean³ showed that pure bismuth salts were toxic. They gave a dog daily 10 grammes of the subnitrate without observing any ill effect, but when the salt was put on a raw surface of any extent, intoxication often followed.

A number of cases of poisoning have been reported when bismuth subnitrate was used and which manifested symptoms of nitrite poisoning alone. In these cases the salt evidently became decomposed and nitrites became formed from the nitrate radicle. This is, of course, a wholly different thing from bismuth poisoning. An instructive report on this point has been made by Jensen.⁴ The symptoms in these cases consist of restlessness, chilliness, weakness and loss of appetite, nausea or vomiting, and headache with a small, weak, irregular but rapid pulse. The temperature is subnormal or fever may rarely appear. There is tingling or numbness in the fingers, dyspnea and cyanosis and great muscular weakness. Methaemoglobin may be produced in the blood by the action of the nitrite. It has been suggested by Jensen and others that in these cases certain bacteria may be especially active in splitting off the nitrate radicle from the bismuth. In bouillon cultures of colon bacilli and of staphylococci (but not of streptococci) it was found that nitrates were formed when bismuth subnitrate was added to the cultures. And the suggestion has therefore been made that the subnitrate should not be used in injecting sinuses, etc., if previous bacteriological examination shall have shown the presence of colon bacilli or

³Lancet, 1913, Vol. 1, April 12, p. 1039. Arch. gen. de. Med., 1887, ii, p. 129.

⁴Loc. cit. Also, Böhme, A.: Archiv. für experimentelle Pathologie und Pharmakologie, 1907, lvii, p. 441.

staphylococci. Perhaps the formation of moderate amounts of nitrites by these organisms in the alimentary canal might be of much less serious import than the production of a corresponding amount in a fistula or sinus. It would seem safer as a rule to use the subcarbonate in preference to the subnitrate.

It has further been suggested by Rost⁵ and Wacker⁶ that the active (therapeutically valuable) portion of Beck's paste may be the vaseline and not the bismuth at all. Some evidence in this direction was apparently obtained by the production of favorable results after the use of vaseline alone, "American" vaseline being more active in this direction than that obtained in Germany, presumably because the former was more impure and irritating. The well-known action of paraffin-related substances in producing skin lesions is referred to by Wacker in this connection.

Under normal conditions it appears that but little bismuth is absorbed from the alimentary canal although occasionally traces may be found in the urine after ingestion of the metal. The excretion occurs mainly through the kidneys and the alimentary canal. In the kidney may be seen that inflammatory condition usually produced when that organ has to excrete metallic substances foreign to the body, the "metallniere," or metal kidney, of German writers. The stools take on a black color which has generally been claimed to be due to the formation of the sulphide of the metal in the bowel, although it has been suggested that this is due to reduction of the subnitrate in the intestine. The sulphide is very insoluble and Meyer and Steinfeld⁷ have maintained that it is precipitated not only within the lumen of the bowel, but also in the capillaries and lymph spaces of the mucosa where hydrogen sulphide penetrates in traces from within the bowel. This causes the formation of minute emboli which clog the capillaries and lead to localized ulceration and gangrene. These authors believed from experimental observations that bismuth was excreted throughout the length of the alimentary canal but mainly in the large intestine where a dense black coloration of the entire thickness of the wall of the cecum and colon could be seen postmortem. This observation reminds one of the excretion of iron. If in addition sulphur preparations were given to animals treated with bismuth salts, then blackening or even ulceration of the mucosa occurred in the stomach and small intestines, also thus indicating excretion of the metal in these regions.

The treatment of bismuth poisoning consists chiefly in removal of the cause. Severe cases of true chronic bismuth poisoning are scarcely seen at present because of the early diagnosis of such cases. Severe nitrite poisoning, however, may occur for it is much more rapid in its onset and may be fairly marked before the patient is seen by the physician. These cases are, however, apparently exceedingly rare. In case symptoms of poisoning from injection of bismuth paste appear, Beck⁸ recommends that the paste be removed as soon as possible. The best way to do this is by washing out the cavity with warm olive oil. The sterile oil is injected and retained for twelve to twenty-four hours, in order to produce an emulsion, which should be withdrawn by means of suction. After

⁵Rost, Franz: *Muenchener Medizinische Wochenschrift*, 1913, ix, Oct. 14, p. 2281.

⁶Wacker, L.: *Muenchener Medizinische Wochenschrift*, 1913, ix, Dec. 2, p. 2674.

⁷Meyer und Steinfeld: *Archiv. für experimentelle Pathologie und Pharmakologie*, 1886, xx, p. 40. Steinfeld: *Wirkung des Wismut*, Inaug. Diss. Dorpat, 1884.

⁸Beck, E. G.: *Loc. cit.*, p. 156. Also *Journal of the American Medical Association*, 1909, Jan. 8. Warfield, Louis M.: *American Journal of the Medical Sciences*, 1912, cxliv, p. 647.

its removal all symptoms should promptly disappear. Scraping out the paste with a scoop is a dangerous procedure, because it opens fresh channels for absorption.

In Röntgen-ray⁵ examinations the ingestion of large amounts of bismuth has sometimes led to the formation of large concretions in the stomach or intestines. Possibly this may occasionally be associated with an increased formation of the sulphide of the metal.

—D. E. J.

The Glucose Content of the Blood

WITHIN the last few years the chemistry of the blood has been receiving increasing attention. Until recently the methods were so complicated and laborious and required such large quantities of blood, that they could not be employed by the clinician. With the growing importance of the subject, however, simplified methods have been devised which permit of repeated determinations, owing to the fact that only small amounts of blood are required, without sacrificing accuracy. Thus Folin and Denis¹ have described methods for the determination of urea, ammonia, non-protein nitrogen and uric acid; Bang, Shaffer,² Kowarsky and others for the determination of glucose; Marriott³ for the estimation of "acetone bodies;" and Bloor⁴ for fat. These methods are now being employed in the clinic. The field is so comparatively new that the data at hand are at present insufficient for definite conclusions in most instances.

The variety of methods for determination of blood sugar is greater than for most other substances of the blood, and as a result of the differing technics employed, comparison of the work of different observers is misleading at times. In any of the methods employed, it is necessary to remove the proteins. This is accomplished with least loss of glucose by combined heat-coagulation and the Michaelis-Rona colloidal iron precipitation, as Shaffer⁵ has shown. In those methods for example, in which the proteins are removed by precipitation with alcohol, there is definite loss of sugar. Shaffer's method is rapid, accurate and requires only 5 c.c. of blood for a determination. Strouse⁶ has recently described a modification of Kowarsky's method which is very similar to Shaffer's, but which is carried out with only 0.5 c.c. of blood. Should it prove to be equally accurate, the smaller quantity of blood needed (which may be obtained from a puncture wound) would be a decided advantage.

With a given method, even though the results may not be absolutely correct, still they are of value comparatively, showing variations of glucose content from time to time in the blood of the same individual, and furnishing a basis for comparing the glucose concentration of the blood of normal individuals with that found in disease.

⁵Cannon, W. B.: *The American Journal of Physiology*, 1898, i, p. 359; 1902, vi, p. 251.

¹*Jour. Biol. Chem.*, 1912, xi, 527.

²*Ibid.*, 1914, xix, 285.

³*Ibid.*, 1914, xviii, 507.

⁴*Ibid.*, 1914, xvii, 377.

⁵*Loc. cit.*

⁶*Bull. Johns Hopkins Hosp.*, 1915, xxvi, 211. (For a comparison of the methods of Bang and Bertrand, see *Fitz. Arch. Int. Med.*, 1915, xiv, 133.)

Studies on the blood of normal individuals have disclosed the fact that diet is of considerable importance. Thus, a value which is normal for a fasting patient may be greatly increased after a meal containing sugar or starch, whereas protein and fat are without appreciable effect on the percentage of glucose in the blood. This fact has been emphasized by Bing and Jakobsen⁷ and by Strouse. The latter also made the interesting observation in a patient with obstinate furunculosis but without glycosuria that the glucose concentration following a meal containing carbohydrates was unusually high; the placing of the patient on a strict carbohydrate-free diet was followed very promptly by cure.

Aside from the response to carbohydrates in the food, Kahler⁸ reports observations which seem to show that there is also a physiological rise in the concentration of blood glucose in women during menstruation.

In disease a few scattered observations are recorded, as yet insufficient to be of great value. Only a part of them are cited below.

Purjesz⁹ finds that with increased activity of the thyroid gland the blood glucose is lower than normal. Bing and Jakobsen,¹⁰ using Bang's method, report similar findings. Administration of the "infundibular part of the pituitary gland" was followed by an increased concentration of glucose. In pancreatic disease Bing and Jakobsen report increased percentage of blood glucose. In Addison's disease Purjesz found a marked decrease in glucose, while Schirokauer¹¹ has found normal values on two occasions in one patient.

Increase of blood glucose has been noted in hypertension. Bing and Jakobsen report cases of nephritis unassociated with hypertension, which also showed an increase in blood glucose content.

Menke,¹² using Bang's method, reports observations on the blood of diabetes. Hourly determinations of the glucose content of the blood showed great variations which, without regard to the severity of the disease, seemed to depend on the taking of food and the activity of the kidneys; a fall in blood sugar was found to be coincident with excretion of sugar by the kidneys. Surprisingly high values were often encountered in fasting patients, but it was Menke's impression that the differences in concentration thus obtained do not furnish an accurate index of the severity of the disease. A comparison of the blood glucose curve after ingestion of like quantities of wheat and oatmeal showed no differences either in the height or rapidity of the rise. Bing and Jakobsen found no direct relationship between the degree of hyperglycemia and glycosuria in diabetes.

The group of cases of so-called renal diabetes is being enlarged through the study of the glucose content of the blood. Novak, Porges and Strisower¹³ have investigated the spontaneous glycosuria of pregnancy. In seven consecutive cases, they found that the glucose content of the blood was not increased, and that the excretion of sugar was largely independent of the diet. With the

⁷Deutsch. Arch. f. klin. Med., 1914, cxiii, 571.

⁸Wiener klin. Wchnschr., 1914, xxvii, 417.

⁹Ibid., 1913, xxiv, 1420.

¹⁰Loc. cit.

¹¹Berlin klin. Wchnschr., 1911, xlviii, 1505.

¹²Deutsch. Arch. f. klin. Med., 1914, cxiv, 209.

¹³Deutsch. med. Wchnschr., 1912, xxxviii, 1868.

carbohydrates eliminated from the food, sugar was still excreted, but the addition of a considerable amount of carbohydrates to the diet did not cause a corresponding increase in the sugar of the urine. Salomon¹⁴ has reported observations on renal diabetes in non-pregnant patients. He finds that the concentration of glucose in the urine is usually under one per cent and that the excretion of sugar is only slightly affected by diet. The blood glucose concentration is usually under 0.1 per cent. After administration of 100 gms. of glucose to the fasting patient, usually only from 1 to 7 per cent of this is excreted in the urine. With the administration of glucose there is a hyperglycemia which persists for a few hours.

It is altogether probable that the results of the study of blood glucose will greatly enrich clinical medicine, not only from the standpoint of more accurate diagnosis and prognosis, but also of treatment.

—R. S. M.

¹⁴Ibid., 1914, xl, 217.

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ORIGINAL ARTICLES

GROWTH *

BY LAFAYETTE B. MENDEL AND THOMAS B. OSBORNE, NEW HAVEN, CONN.

THE solution of the chemical problems of growth has been approached along various avenues. One of these, and perhaps the earliest to be followed by any considerable number of investigators, leads to the *chemical analysis* of both growing and fully grown individuals. Its primary object is to disclose the characteristics of growth by a comparison of the chemical composition of organisms at various stages of their development. Despite the considerable abundance of data thus accumulated, relatively little of major importance has been contributed thereby to our knowledge of the subject. Growing cells and tissues may be morphologically and functionally unlike the fully developed structures into which they are finally transformed, but the analytical methods of the chemist of to-day are not sufficiently refined to reveal any eminently unique features of make-up characteristic of growth.

It is true that embryonic and other growing tissues are comparatively rich in water, and that the water-content diminishes to a certain degree with increasing age; but aside from such rather inexpressive generalizations respecting quantitative variations in composition at different stages of growth, no striking facts have yet been furnished by the devices of tissue analysis. Equally unprogressive has been the study of the comparative biochemistry of growth in the direction of qualitative analysis. From a purely biological standpoint it is of interest, and a result perhaps to have been anticipated, that there is a fixity, so to speak, in the composition of animal structures at various sizes of the same individuals. The number of the cells or the volume of each of them may be increased enormously; yet, broadly considered, the available analytical data show no striking differences in the chemical organization of the resultant protoplasmic mass in comparable tissues at various periods. In other words, aside from transitory depositions of reserve materials such as glycogen or fat, muscle remains alike in

*From the Sheffield Laboratory of Physiological Chemistry in Yale University and the Laboratory of the Connecticut Agricultural Experiment Station at New Haven, Conn.

its gross composition, and the nervous substance exhibits essentially the same chemical characteristic components, independent of age, diet, or environmental condition.

Several years ago a study of the composition of the bodies of mice kept upon diets of widely different types led one of us to express the outcome in these words: "The constant composition of the organism which is exhibited by our analysis, does not speak in favor of the possibility of depriving the body by alimentary procedures of any constituent excepting fat that is essential for its functions. On the contrary, it appears that the organism adheres to its proportionate composition. A deficiency in the diet, a lack of some food component, is not responded to by growth in which the tissue produced is chemically abnormal and shows a depletion in the missing factor. Its composition remains unaltered. Normal growth can proceed only when all the important constituents are assimilated in the proportions in which they make up the body. Losses are sustained only by the *uniform* disintegration of the tissues, whereby their relative composition remains unaltered."¹

A more effective advance upon the chemical questions relating to growth has been made by the study of *nutrition* in this period of life. This more modern plan has meant a determination of what constructive units are essential for the building up of an adult organism, what materials must be furnished to the growing individuals, what possibilities of synthesis are inherent in them and will enable them to supply by construction the necessary tissue components. The successful pursuit of this method of inquiry depends upon the justifiable assumption, already mentioned, that protoplasm, if it is constructed at all, is not made into a fundamentally defective variety of diet, though deficiencies in the latter may lead to an unmaking of cells already developed.

The questions relating to the initiation of growth and to the fundamental energy factors are not touched upon in this review. Certain modifying features of nutrition in growth are more or less obvious. The necessity for certain elements, like calcium, calls for no further comment here than to inquire to what extent the need of them is specific in growth. In the ordinary wear-and-tear of life, or what is technically designated as the maintenance metabolism, losses of structural elements call for restitution. In the case of the most of them we shall probably not err widely in saying that the metabolism of growth represents a highly exaggerated instance of the same needs, but on a scale appropriate to produce increment instead of mere maintenance of body substance.

Modern chemical physiology has demonstrated in a convincing way that the diverse proteins which are now known to occur in nature can no longer be considered solely in a generic way in the roles which they play in the organism. These nitrogenous compounds lose their biological identity, even before they leave the alimentary tract, by becoming disintegrated into the chemical units—the amino-acids—out of which the proteins are built up. In so far as these amino-acids merely serve as fuel, it seems to matter little what their precise nature is. All of them can be, and usually are, destroyed readily in the metabolism. Inasmuch as tissue proteins are broken down in a small, yet seemingly inevitable degree in the wear-and-tear, the problem of satisfactory restora-

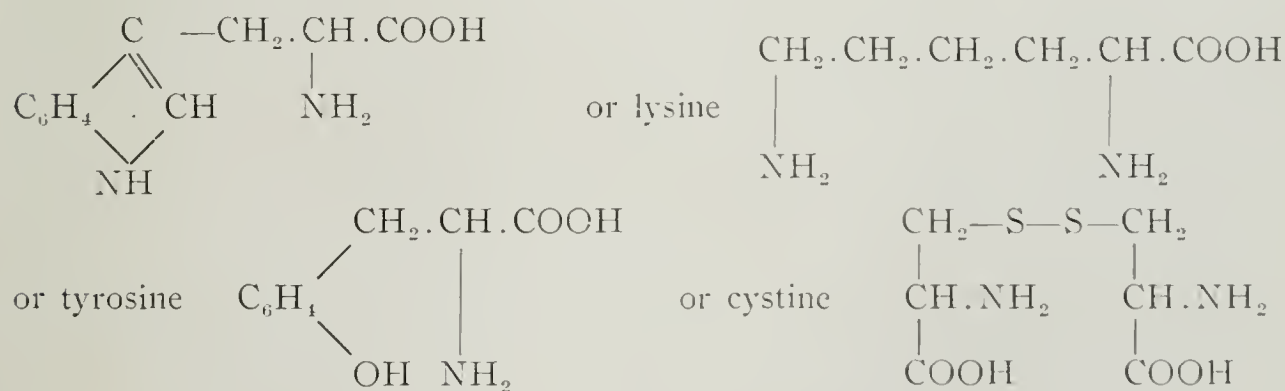
¹Mendel, L. B.: Der Einfluss der Nahrung auf die chemische Zusammensetzung des Tierkörpers. *Biochemische Zeitschrift*, 1908, xi, 281.

tion arises. During growth additional new protein molecules are to be provided. At the present time it may be said with confidence that many, though not all, of the amino-acids must be offered ready-made in the diet, because there is no evidence that they can be synthesized *de novo* even in growing mammals. For the lower forms of animal life this statement may not be valid, as it is not in the case of plants. In the higher forms, however, the exhibition of dietary proteins which are deficient in respect to their yield of any of the essential amino-acids may be expected to lead to suppression of growth and even failure of maintenance, depending on the degree and nature of the structural shortcomings.

The evidence in support of this general statement has resulted from the possibility of preparing a ration which is complete or adequate for growth in every respect other than the protein component. This latter factor can then be added experimentally in any way desired. The casein of milk is one of a few pro-

teins which fail to yield the amino-acid glycocoll $\begin{array}{c} \text{CH}_2\text{COOH} \\ | \\ \text{NH}_2 \end{array}$ upon analysis.

Tissue proteins, and particularly those of the omnipresent connective tissues, include a relatively large content of the glycocoll group in their make-up. Yet animals can be grown to maturity and into a second generation upon a diet containing the glycocoll-free casein as practically the sole source of food protein. Here, then, is a striking illustration of the capacity of a growing organism to supply, by synthesis, one of the essential amino-acid constructive units of its protoplasm. On the other hand, there are proteins, particularly of vegetable origin, which fail to furnish one or more of the amino-acids, such as tryptophane



which tissue proteins also yield. As the result of very recent experimental investigation there is little room for doubt that all of these mentioned amino-acids must be furnished ready-made to an organism for the constructive uses of growth. The organic chemist will appreciate why the laboratory of the living cells may be unable to synthesize nuclei so complex as those of tryptophane or tyrosine and yet be competent to produce a comparatively simple product like glycocoll.

The best proof of the indispensability of some of the amino-acids has been furnished by actual feeding experiments. Without the proper complement of these units, even the best diet spells disaster; and the addition of the missing ones, either as such or in the guise of proteins containing them, brings renewal of growth proportionate, as a rule, to the extent to which the deficiencies are made good quantitatively as well as qualitatively. The lack of an essential pro-

tein factor need not be an absolute one to produce malnutrition. It has become apparent, as Abderhalden pointed out long ago, that where the organism cannot synthesize an essential fragment, the nutritive pace is set, so to speak, by the minimum of any indispensable dietary ingredient. In practice this means that however abundant in protein and seemingly "balanced" a diet may be from the standpoint of the theories of nutrition of a generation ago, it is certain to be inadequate for growth if the proteins are qualitatively unsuitable as in fact they may be if derived from a single source.

For the problems of practical dietetics and particularly of animal production it is further of interest to note that chemically identical proteins are apparently not present in animals or plants of different species unless they are biologically closely related. In so far as the differences are an expression of inequalities in the amino-acid make-up of the protein from these varied sources they are likely to have significance in the construction of a dietary suitable for growth. There are various protein by-products, for example from the cereal, milk and meat industries, which are available as low priced nutrients and the successful use of which must henceforth be determined, in so far as they enter largely into the rations of growing animals, by the standard of their competence to furnish the essential amino-acids suitably. It has been shown that unquestionable differences exist in the economy of correcting various inadequate protein foods with appropriate supplementary proteins. The problem seems in part at least to be one of suitably combining products that are deficient in one or more nutrient units so that the mixture shall not have any serious relative shortage of any one of them.

The upshot of these newer developments in the physiology of nutrition has been to give a new trend to the biochemistry of the proteins and to lend a greater importance to this pre-eminent group of food stuffs. Those whose field of interest lies far away from these strictly chemical aspects of nutrition will appreciate the comment of one of our colleagues (von Fürth) when he says: "I invariably experience a feeling of envy when I read the letters of Liebig, Wöhler, or Berzelius, and note how important an event for these fortunate individuals was the appearance of every scientific publication. With what devotion and joy they read and re-read every detail of even the smallest scientific contribution. Today, owing to the great mass of scientific literature, we are in danger of losing the naive pleasure which anything novel should afford; and we are likely to sacrifice the spirit of inquiry or inquisitiveness that attaches to the soul of the man of science. And so, today when the devotee of biochemistry, working in the sweat of his brow, is barely able to orient himself in the essentials of its literature, familiarity with its content has long since become an impossibility for him who stands apart from any immediate concern with such topics." (v. Fürth: *Probleme*, i, p. 2.)

And now, at a time when the energy problems of growth have reached a degree of solution that gives a clear and fairly comprehensive insight into this aspect of nutrition, so that this chapter of physiology can at length be reviewed with a fairly satisfactory understanding of what it involved, entirely new questions have thrust themselves into the foreground. Physiologists have long realized that the ideal way to study the problems of nutrition would be by feeding

artificial mixtures of the isolated and purified nutrients. The successive failures of the attempts in this direction, which need not be detailed here, coming in conjunction with evidence from other sources, have awakened us to the realization that perhaps something more than the familiar proteins, fats, carbohydrates and inorganic nutrients are essential to the persistence of the life processes or the accomplishment of prolonged growth. The modern researches on the physiological significance of the secretions of the ductless or so-called endocrine glands and the growing evidence for the regulatory function of hormones or chemical stimulants distributed by the circulation have made it easier to believe that substances which are neither significant as sources of energy nor sufficiently abundant to construct new portions of protoplasm may nevertheless exercise a dominant effect upon nutrition. To one who has been brought up in the study of exact stoichiometric relations in science there is something almost unscientific and mystical in the discovery that mixtures of food stuffs which are selected from the chemist's supplies and fail to maintain animals can be made adequate by the addition of milk, for example, in quantities far too small to have significance as a source of energy. We are dealing here with what have been called food accessories or "vitamines."

Growth appears to be in some degree dependent upon the presence of chemical determinants of this order. For the present they must not be confused with other known essential nutrients, with suitable amino-acids, or inorganic salts, or appropriate carbohydrates. It must be recognized, at the outset, that unless the conditions are suitable for maintenance, growth which is normally superimposed upon it, cannot proceed. The value of food accessories of as yet unknown chemical nature has been made evident of late by feeding experiments with isolated food substances, where additions of small amounts of naturally occurring products have prevented nutritive decline or brought restoration. This is notably true of the "protein-free milk" devised by Osborne and Mendel for feeding rats. No artificial imitation of this natural mixture, which contains milk sugar, inorganic salts and very small quantities of unknown ingredients, has been devised to replace it satisfactorily for considerable periods of growth. The success of the "natural" product seems to be dependent upon the presence of undiscovered "determinants" in minute traces. Evidence for the existence of such accessories is further furnished by the so-called deficiency diseases. In some cases heating seems sufficient to destroy some thermolabile determinant of maintenance. The development of scurvy from the use of heated foods is an illustration of this point.

It is not unlikely, in the light of the meager data now at hand, that there is more than one determinant or food accessory that promotes suitable maintenance. There is no justification whatever for including these unknown factors today in a common chemical group aside from the fact that they seem to be essential and to act in small amounts in ways not hitherto taken cognizance of. Some of them are unquestionably quite thermostabile, others are perhaps thermolabile. With the possible exception of Funk's beriberi "vitamine" they have hitherto eluded chemical identification. Perhaps they merely stimulate the appetite and thus induce an inadequate food intake; though this explanation seems to be clearly contradicted by some of the existent reports. Perhaps they merely

supply cell adjuvants like iodine or manganese or some organic compound, the need of which we have overlooked because it is quantitatively so small. In any event they represent an undetermined factor that must be reckoned with.

However, an adolescent animal may actually fail to grow upon a diet which serves well to maintain an adult individual of the same species. Grown rats have been kept in good health for many months on a ration consisting of protein, sugar, starch, "protein-free milk" and lard. Upon this diet the young of the same species grow for a limited period and then invariably decline. We have had the almost paradoxical experience that those animals which grow on the diet mentioned presently die, whereas those which do not experience growth are more likely to continue to live. This may be interpreted to indicate that in growth an essential substance presumably stored in some measure in the adolescent organism is used up, whereupon nutritive failure ensues; whereas in the absence of active growth a depletion of the material so necessary does not take place in the same degree. It has been shown that this cessation of growth can be stopped and growth resumed by the substitution of other naturally occurring fats for part of the lard of the ration. In butter-fat, egg-fat, cod liver-fat, and beef-fat,—and more specifically in the fractions of these containing the oil components liquid at ordinary temperatures—there exists a determinant of growth in the sense in which this expression has been discussed above. It is apparently not a nitrogenous or phosphorized compound like the phosphatides which have been charged with growth-promoting effects, but experimentally its action is pronounced in rats. When other factors, such as suitable proteins or salts, are missing the chemical accessories yielded by the natural fats are obviously incapable by themselves of insuring growth.

How or why do these chemical determinants promote growth? The best that we can do today is to recite the facts. Perhaps they promote appetite and lead to gain of weight by inducing suitable food intake. It is not easy to reconcile such a hasty explanation with the marked differences between the fats studied. Perhaps they merely represent chemical ingredients necessary at all times for the body cells, but needed in great abundance during increment in size and therefore not available in sufficient amounts in a diet adequate for mere maintenance with limited wear-and-tear or tissue loss. These problems are still with us.

THE GENERAL PATHOLOGY OF PELLAGRA, WITH SPECIAL REFERENCE TO FINDINGS IN THE THYROID AND ADRENALS*

BY PLINN F. MORSE, M.D., DETROIT, MICH.

PELLAGRA is interesting from the standpoint of pathology because it bears a rather unique place among the well-known clinical conditions. No disease has been more thoroughly studied from most standpoints and less thoroughly from that of its general pathology. The incidence, geographical distribution, mortality, relations to diet and to possible infective sources, have been the subject of extensive investigation by many organized commissions and independent observers, but perusal of the reports from these sources gives one the impression of marked one-sidedness in the method of attack, for the reason that there seem to be no recent examinations by modern methods of the pathological anatomy of the disease as a whole. All the recent complete studies have been focused especially on the nervous system, and Harris states that he has no doubt but that pellagra will be found to be essentially a nervous disease when the problem is finally solved. Such excellent studies of the histopathology of the nervous system appear in the literature that it is not necessary to do more than review them. Concerning other organs and tissues however very little has been said recently. The bibliography up to 1910 and the pathological findings up to that time are reviewed by Lavinder and Babcock in their translation of Marie's book. Nothing has been added since except more detailed studies of the nervous system.

The pathological findings up to 1910 as abstracted from Lavinder and Babcock's review of the literature are briefly as follows:

Central Nervous System.—Pigmentary, granular, and fatty degenerations of the nerve cells, occasional loss of myelin around the axis cylinders, varying degrees of degeneration and sclerosis of the posterior and the lateral pyramidal tracts. The degeneration in the posterior columns differs from tabes in certain important respects. The increased pigmentation of the posterior root ganglia described by Lombroso is one of the most important constant changes. Belmondo, cited by Lavinder and Babcock, naively states that this pigmentary change occurs even in the "youngest cells of the ganglia," not recognizing the fact that all nerve cells are of prenatal origin. A large number of inconstant concomitant changes common in many other conditions, such as hemorrhage, intercurrent myelitis, osteomata of the arachnoid, thickening of the meninges and adhesions to the skull cap, myelin droplets in various parts of the cord, etc., are described by various authors in detail.

Arteriosclerosis of the vessels of the central nervous system and its results have been frequently noted.

Lungs.—Accidental complicating lesions such as emphysema, pneumonia, edema, congestion, tuberculosis.

Heart.—The constant and striking change has been extreme brown atrophy

*From the Buhl Memorial Laboratory of Harper Hospital, Detroit.

of the heart muscle. Hypertrophy, atrophy, atheroma, hydropericardium and aneurism have been occasionally met with.

Liver.—Brown atrophy was the most constant change, congestion and fatty infiltration were sometimes found.

Spleen.—Atrophy.

Kidneys.—Atrophy, fatty changes in the tubules, and increase of interstitial connective tissue.

Intestines.—Muscular atrophy of the wall, hyperemia and ulceration of the rectum. Other changes, such as anemia and hyperemia, chronic enteritis, and thickening of the Peyer's patches have been accidental and less constant.

Suprarenal capsules, pancreas, and testicles are reported as normal. No definite references to microscopical appearances are made.

Female Sexual Organs.—These showed many noncharacteristic lesions of common occurrence in other conditions.

Muscular System.—Atrophy was the most constant finding.

Skeleton.—Fragility of the ribs and other bones dependent upon eccentric atrophy was frequently observed along with evidence of increased blood destruction in both the marrow and the spleen.

The most complete study of the central nervous system in pellagra made in this country is that of Harris who confirmed the work of Babes, Scion, and Marinesco and added certain important features. Harris described small collections of lymphocytes in the brain and swelling of the neuroglia in association with the vessels. Hitherto undescribed changes in the Purkinje cells probably explaining the ataxic form of the malady were brought out by him. A more recent minute study of the nervous system in pellagra has been made by S. A. K. Wilson, who lays stress on the degeneration of π granules of Reich, and marked degeneration of the cells in Clark's columns. Wilson believes that the degenerations found in the cord are pseudo-system degenerations.

Skin.—The findings in the skin are not reported as very striking or characteristic. There has been a rather constant increase of the horny layer with marked desquamation, the stratum Malpighii has been found atrophic, and the corium sclerotic with thickening of the vessels, and perivascular infiltration. Gurd has described these changes in detail and has considered, as others have before him, the changes due to the action of actinic rays upon abnormally sensitized skin.

Specific references to the ductless glands are rare. Harris and Wilson both state that examinations of the ductless glands in their cases were made and the organs found normal.

An interesting clinical finding of great importance is the occurrence of a lymphocytosis as reported by Hillman and Schulle, and others.

The clinical history of the patient from whom the material here reported was obtained is briefly as follows:

Mrs. W—, Widow, age 38, housewife, English, admitted to Harper Hospital on September 24, 1915, on the service of Dr. Haass.

Patient complained of weakness, diarrhea, "sore arms" and "cloudiness in head," the duration the patient stated to be about four months. It was very difficult to obtain an adequate history on account of the patient's marked mental

torpor bordering at times upon amentia. Family History. No clear statements obtainable relative to diseases or cause of death of parents or other relatives. Personal History: Husband died three years ago of tuberculosis. One child twelve years of age, living and well. She has worked for some time in a chandelier store as scrub woman. Came to this country two years ago. Patient cannot remember any other sickness in her life. Has not menstruated for seven months.

Present Trouble.—Has not been feeling well for last seven months since she stopped menstruating. Her appetite has been poor with an occasional headache and dizziness. For the last four months patient has had a diarrhea constantly. She has been bothered by "cloudiness and haziness in the head," poor memory, irritability, headache, weakness, and dizziness. A skin lesion appeared four months ago, first between the fingers on the backs of the hand; it gradually spread involving the hands and extensor surfaces of the forearms, the knees, feet and neck then became involved to a less extent. The patient dreads food and even liquids because they nauseate her and give her diarrhea. She is unable to taste anything and her mouth is dry and sore. She sleeps poorly and of late has lost control of her rectal sphincters. (Ulceration?)

Physical Examination.—The patient is a medium built woman with dull expression and staring eyes. Poorly nourished scalp with hair rapidly thinning. Pupils equal and react to light and accommodation. The lips are dry with corners eroded and are partly covered with a silvery gray coat. There are erosions of the mucous membrane of the mouth and the tongue is reddened and swollen. There are no palpable glands. Heart and lungs, negative. Abdomen, tender and flaccid; liver and spleen, not palpable. On extensor surfaces of the hands and forearms the skin is thickened, cracked and pigmented, with deep wrinkles, resembling the skin of a very old person except that it is thick, hard, dry, and pigmented. The skin is so much thicker in the portion involved that taken with the distribution the lesion gives the impression of a glove. The coloration varies from a narrow zone of bright pink erythema around the edge to dark brown in the thinner portions of the lesion and a brownish-yellow in the thick leathery expanse of the lesion proper. The same condition is present over the knees, on the backs of the feet near the toes, and around the neck in the manner of a pendant. The reflexes are sluggish and sensation is delayed especially over the involved area. The patient has not been able to stand on her feet for several weeks probably because of weakness.

White cell count.—7,300 Polys., 53%; Small lymphocytes, 38%; Large lymphocytes, 6%; Transitionals, 3%; Hemoglobin, 75%.

Wassermann.—Negative. Urine negative. Temperature very irregular, varying from 97 F. to 101 F. Examination of stools on warm stage revealed no amebae or other parasites.

A diagnosis of pellagra was made by Dr. Haass and the patient referred to the dermatological service of Dr. H. R. Varney who concurred in the diagnosis.

It later appeared that the patient has been at the Woman's Hospital and turned over to the Poor Commission by Dr. Vernier as a pellagrin. The Poor Commission sent her to St. Mary's Hospital where the diagnosis was made by a house physician who had resided in the South. The case was immediately deported from

St. Mary's Hospital by the Sister in charge under the impression that pellagra was contagious. She was then sent to Harper Hospital and was later examined by Lieut. Weber of the Public Health Service who concurred in the diagnosis and was arranging for her deportation as a diseased alien when she died. It is therefore thought that the characteristic combination of skin lesion, gastrointestinal disease and profound mental disturbance coupled with the confirmation of several independent diagnoses made by various physicians all of whom have had experience with pellagrins, make the diagnosis certain.

The patient died on October 4th at 10 a. m. Autopsy was performed at 3 p. m. of the same day.

AUTOPSY NOTES.

The body is that of an emaciated female apparently about 40 years of age. There is moderate overgrowth of hair over the body with somewhat of a masculine type of distribution. Eyes and nose negative. Mouth shows marked pyorrhea and a bluish line on the gums somewhat resembling that of lead poisoning. The buccal mucosa is ulcerated, the tongue red at the margins, dry and cracked, with a heavy yellowish coat toward the center. Both hands and arms present marked skin changes. On the extensor surfaces it is very thick and wrinkled with marked pigmentary deposit. The outer borders of the lesion are dark brown in color and the rest a brownish-yellow. The skin appears edematous and thickened and is extremely wrinkled. There is increased desquamation. The lesion ends on the extensor surface toward the elbow in a rather "V" shape. Both knees show the same lesion. The backs of the feet near the toes show less advanced lesions there being not so much thickening and more desquamation. Around the neck somewhat in the shape of a turned-in shirt the lesion appears with patches of more intense involvement here and there along its course. Section shows the fat of the abdominal wall to be deeply pigmented and yellowish. The muscles are dry and pale. There is no gas or fluid in the peritoneal cavity. The liver is five finger-breadths below the costal margin in the mammary line. The omentum is congested, the appendix retrocecal and somewhat dilated. Superficial topography of the abdomen otherwise negative. Thorax: The sternum is narrow; the bony prominences at junction of the costal cartilages and ribs somewhat elevated. The sternum is very rich in deep red marrow. The pleurae are free from adhesions and there is no fluid. The heart is dilated; the apex is two inches outside the mammary line at the sixth interspace. Small amount of amber fluid in the pericardium. Heart negative over outer surface. Left ventricle empty, mitral admits two fingers. The muscle of the left ventricle is thinner than normal, deep brown in color and the endocardium shows well-marked fatty striations. The mitral flaps are thickened and somewhat shortened but present no other abnormal appearances. The right ventricle contains a postmortem clot; the wall is thin and similar in color to the left. The tricuspid admits four fingers, but the flaps are normal. The pulmonary artery contains a postmortem clot. The semilunars of the pulmonary are negative. The first portion of the aorta shows a moderate linear sclerosis. The semilunars of the aorta are normal. The left coronary is very markedly sclerosed, showing nodular and linear thickenings with narrowing of the lumen

throughout its extent. The systemic aorta presents an especially marked patch of sclerosis at the celiac axis. The heart weighs 175 grams. The right lung weighs 325 grams, crepitates throughout, is moderately anthracotic and dry on section. At the root are calcified anthracotic lymph nodes (healed tubercles). The left lung is similar to the right but is more anemic; weighs 220 grams.

Neck Organs.—Pharynx and esophagus normal. Larynx negative. The trachea presents a sero-mucous exudate. The tongue is thickened, dry, reddened and cuts with more resistance than usual. There are at first apparently no thyroid lobes, but careful dissection reveals two fibrous plates, about a quarter of an inch thick, which occupy about the surface area of the normal thyroid and have its position. Both of these plates taken together, upon being dissected out weigh ten grams. No parathyroid bodies can be found in this dense mass of connective tissues.

Liver.—Weight 1,550 grams. The surface shows an irregular yellowish mottling. On section it offers increased resistance to the knife and has a distinct fatty shine. *Adrenals:* The adrenals show marked hypertrophy. Their combined weight is 27 grams. *Kidneys:* The left kidney weighs 135 grams, is quite soft and swollen, dripping blood freely. The right kidney weighs 175 grams and is similar to the left. *Gastro-intestinal Tract:* The gastric mucosa is congested and shows some small erosions and petechial hemorrhages. The pyloric region is normal. The duodenum shows no lesions beyond congestion. The small and large intestine show no lesions except that there is marked post-mortem dilatation of the small and large bowel with thinning of the wall. There is a large area of ulceration with granulation tissue from the internal sphincter to the anus. *Pelvic Organs:* There are old adhesions between the sigmoid, uterus, and adnexa.

Brain and Cord: The skull-cap is normal. No thickening of the dura is observed and the sinuses contain only a little postmortem clot. The brain presents no gross changes; there is no apparent thickening of the pia mater and the convolutions are normal. The base of the brain shows no abnormalities. (The brain was put in formalin and further section done about two weeks later without any gross changes being disclosed.) The cord presents no gross changes.

MICROSCOPICAL FINDINGS.

Adrenal.—Sections of the adrenal glands were fixed in formalin and Zenker's fluid, and longitudinal and cross-sections stained in hemotoxylin and eosin. The relations of cortex and medulla are about normal. The medulla presents a beginning postmortem digestion. The cortex shows normal relations of the glomerular, fascicular, and reticular zones. There is perhaps some decrease in the prominence of the fatty droplets of the cortical cells. The medullary zone is remarkable for the marked dilatation of the blood sinuses and of the small capillaries (Plate III, Fig. 7). The chromaffinic cells are somewhat reduced in number and there is a very moderate fibrosis of the medulla. Around all the large vessels and also scattered diffusely through the adrenal medulla, we find focalized collections of lymphocytes (Plate III, Figs. 8, 9, 10). These show a definitely infiltrative chronic inflammatory reaction with a few plasma cells (Plate

PLATE I.

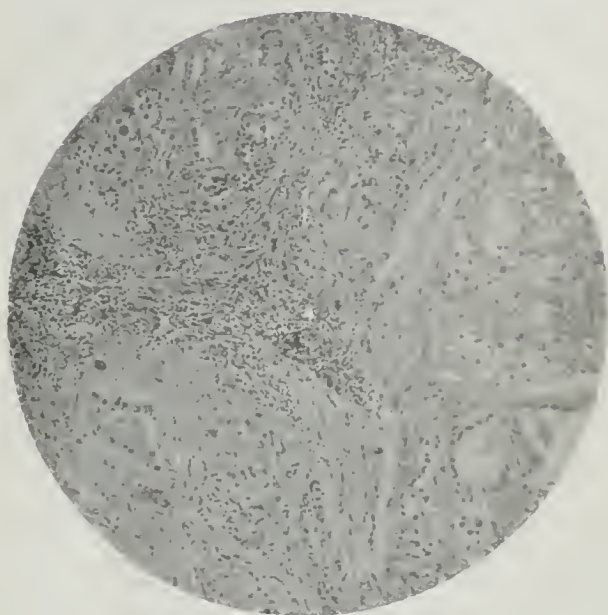


Fig. 1.

Thyroid, showing sclerosis and round-celled infiltration. Area of adenomatous gland at margin.

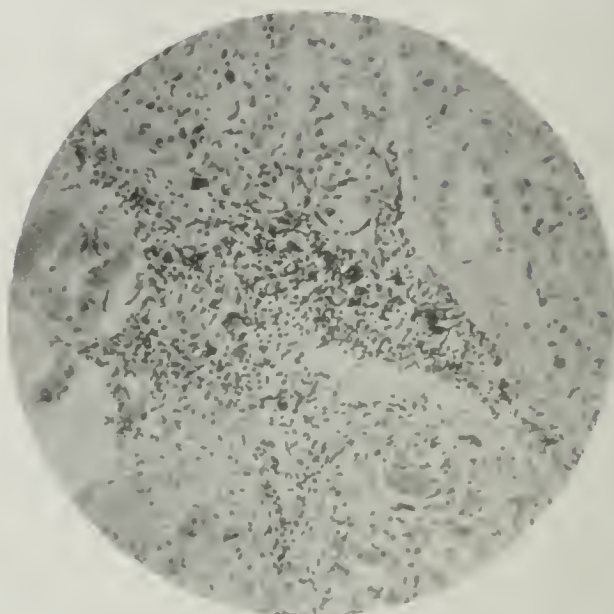


Fig. 2.

Thyroid, high power, showing round-celled infiltration.

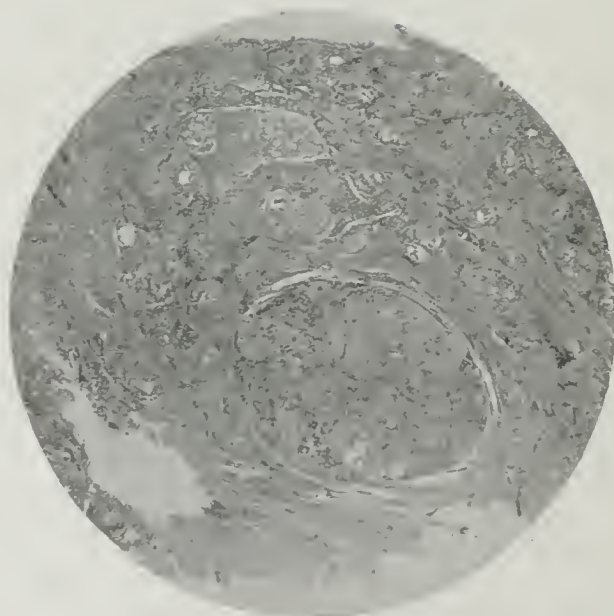


Fig. 3.

Thyroid, low power, showing widespread sclerosis of gland.

PLATE II.

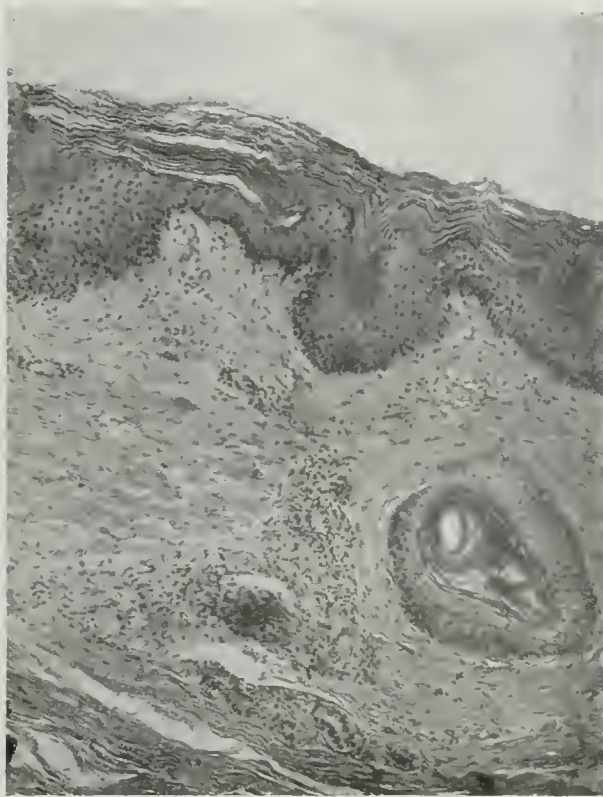


Fig. 4.

Skin, high power, showing infiltration and sclerosis. Margin of active lesion.



Fig. 5.

Skin, low power, center of old lesion.

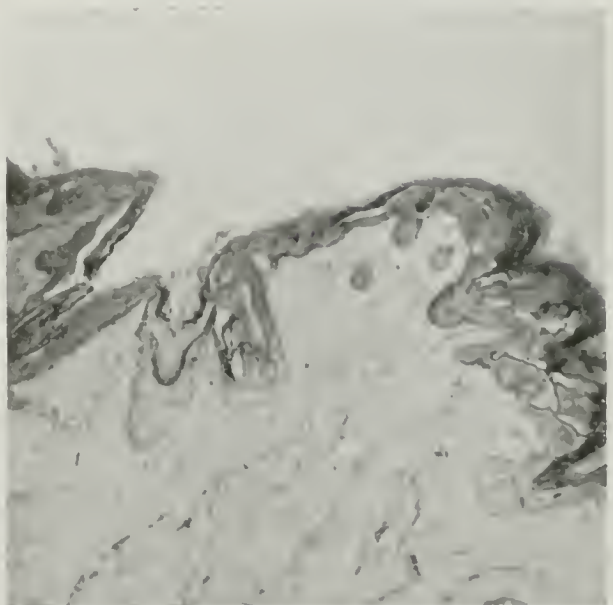


Fig. 6.

Skin, old lesion.

IV, Figs. 11 and 12). The adrenal presents the general picture of moderate atrophy of the medulla with practically no changes in the cortex. In this atrophic medulla we find great dilatation of the blood-spaces and round-celled infiltration focalized especially around the blood vessels.

Heart.—Sections made from both ventricles show moderate hypertrophy of individual muscle cells with a subsequent brown atrophy of extreme degree. There is irregularity in the size and staining properties of the nuclei. The heart muscle is engorged with blood and all the small capillaries are dilated. One area of myocardium has undergone anemic infarction (Plate VI, Fig. 18). The cells are dead but there is no connective tissue reaction as yet. This is to be interpreted as a recent event due to the very advanced sclerosis found in the left coronary artery. The endocardium is moderately but diffusely thickened throughout. This thickening consists merely in the laying down of connective tissue, probably representing a senile or nutritional change; there is no round-celled infiltration or other sign of active endocarditis. The most striking change found in the heart is the extreme degree of brown atrophy, a condition which has been noted before by Lombroso and others. Sections of the blood-vessels show throughout a more or less marked degree of sclerosis. It is interesting that the sclerotic changes in the left coronary artery (Plate VI, Fig. 19) far outstrip those of any other vessel. The sclerotic changes of the aorta consist of a moderate diffuse fatty change beneath the intima, with an occasional erosion of the surface. There is very slight connective tissue reaction. The elastic membranes of the aorta do not seem to be greatly reduced in number. The diffuse bluish cast of the whole media would suggest an early stage of calcification without any definite areas being present. No round-celled infiltration appears, either in the media or in the vascular areas of the adventitia. The sclerosis of the coronary has gone on to an advanced stage. There is extensive calcification of the media with large calcified plaques extending for some distance along the wall of the vessel. Both the left coronary and its branches are extensively calcified.

Thyroid.—The changes in the thyroid are more striking than those of any other organ of the body. There is practically no thyroid tissue present that might be considered in any sense normal. The gland consists of a mass of new and old connective tissue (Plate I, Figs. 1, 2, 3) infiltrated with round-cells, through which, interspersed here and there are islands of thyroid tissue either being destroyed by the pressure of the surrounding connective tissue, or proliferating and adenomatous acini in small encapsulated areas like those found in old degenerating goitres. These follicles contain no colloid but are gland acini with columnar or high cubical epithelium; only very rarely is seen an atrophic follicle with a small mass of shriveled colloid. There is one area of this adenomatous tissue slightly larger than the rest and well encapsulated. The amount of glandular tissue as a whole is extremely small. The picture presented by this thyroid is unique in that it is a true chronic interstitial productive thyroiditis. There is marked increase of connective tissue, old connective tissue showing hyaline change, and areas of young connective tissue around areas of extreme round-celled infiltration. This inflammatory reaction has gone to the point of almost total glandular destruction, so that only small islands of gland-

PLATE III



Fig. 7.

Adrenal, low power, showing dilated blood spaces and fibrosis of medulla.

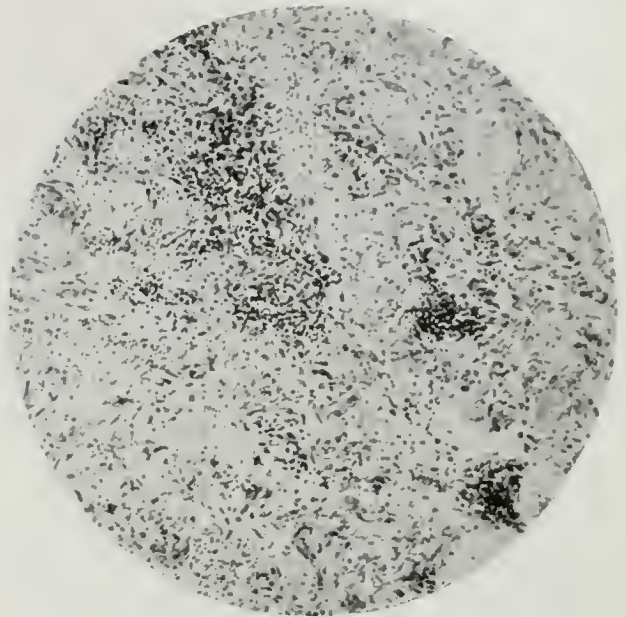


Fig. 8.

Adrenal, infiltration in medulla with fibrosis.

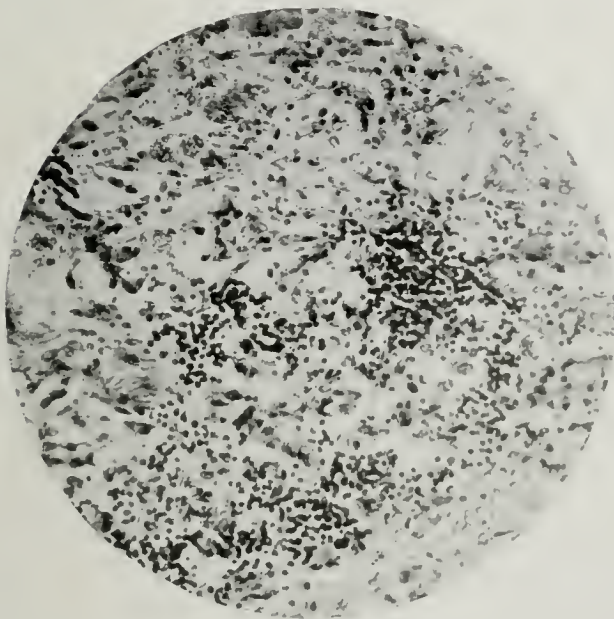


Fig. 9.

Adrenal, high power, showing infiltration in medulla.

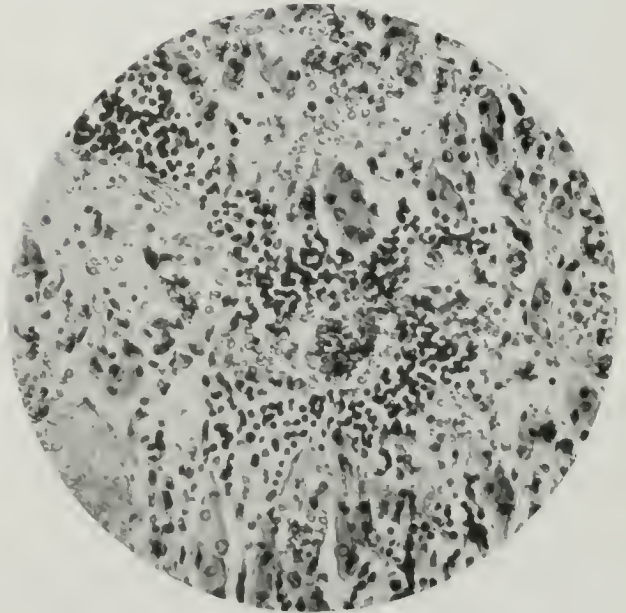


Fig. 10.

Adrenal, chromaffinic tissue associated with round-celled infiltration in the medulla.

ular tissue are left, and they, probably in an effort to compensate for the embarrassed glandular function, have taken on abnormal appearances. No thyroid ever examined by the writer has ever presented this picture. It is not the picture of any of the well recognized types of thyroid disease. The round-celled infiltration consists almost entirely of lymphocytes but plasma cells are occasionally found. The picture suggests not so much an infective process as the reaction to some chronic intoxication with gradual destruction of the gland by the inflammatory reaction, inasmuch as the usual picture of an infective type of inflammation is absent, and we get rather the idea of a diffuse sclerosis due to some diffused chemical irritant, than of infection with its focalized manifestations in the organ. The picture reminds one of an old sclerotic kidney except that the process is more active as is evidenced by the round-celled infiltration.

Hypophysis.—The hypophysis as a whole is larger than normal. Sagittal sections of the hypophysis were fixed in formalin and in Zenker's fluid, and stained in hemotoxylin. Microscopical examination reveals nothing of interest, except perhaps some increase of the colloid follicles of the pars intermedia. The pars anterior shows the normal basophilic cells in the normal proportion. The large blood sinuses are engorged with blood. There is no round-celled infiltration. The capsule is not thickened, and there is no adenomatous hyperplasia of the cellular portion. The pars nervosa is about normal in extent and presents no abnormalities of structure.

Gastrointestinal Tract.—The stomach presents no striking abnormality. There is a disappearance of the acidophilic cells and prominence of the mucous forming acini. The muscularis shows no marked atrophy. There is a moderate congestion of all the coats. The lymph follicles are normal in size and number. There is no erosion of the surface, no ulceration and no round-celled infiltration, in the sections examined. Except for the marked disappearance of the acidophilic cells, the stomach must be considered normal. Passing into the duodenum we find the thickness of the wall fairly normal. There is marked congestion, especially of the vessels of the villi. The epithelium of the villi is all gone (postmortem change) and the villi appear more fibroid than normal. There is no evident atrophy of the muscularis and no round-celled infiltration anywhere in the duodenal portion of the intestine. Brunner's glands are normal in appearance. Farther down the small intestine the same conditions are noticed; the villi are hypertrophic, fibroid and congested and totally eroded of epithelium, except for the bases of the crypts. There is no marked atrophy of the muscularis as is described by most writers and the brown pigmentation is much less prominent than some reporters have found. The lymphoid apparatus of the intestine is rather atrophic. Contrary to the findings of some other observers, the Peyer's patches are atrophic and possess few germ centers. No ulcerations were found anywhere in the intestinal tract, except at the anus. The large bowel presents the same changes as the other portions of the intestinal tract (Plate VI, Fig. 20), except that there is more extreme stasis here than in any other portion of the gut. The fibroid change of the mucosa is also somewhat more marked. There is the same erosion of epithelium. No definite atrophy of the muscularis can be made out. The lymph tissue is also somewhat atrophic. There is increased mucous formation and cystic change in portions

PLATE IV.

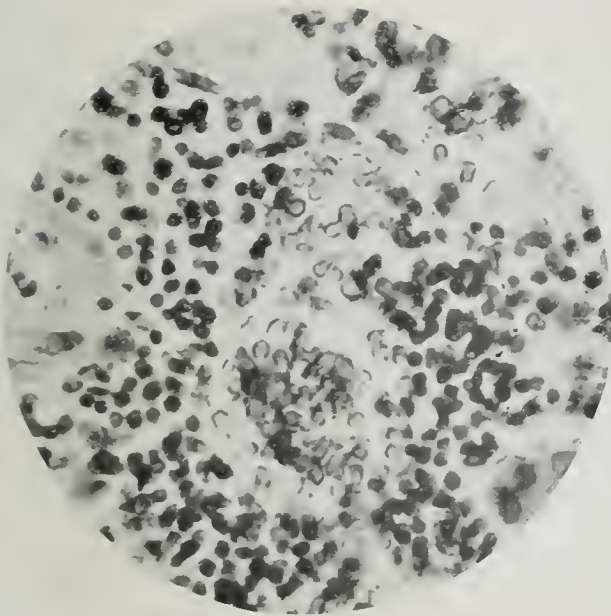


Fig. 11.
Adrenal, high power, perivascular infiltration
around a capillary.

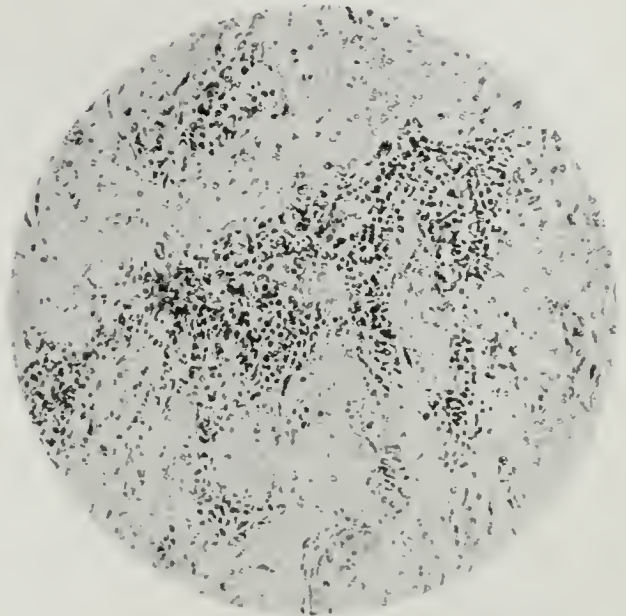


Fig. 12.
Adrenal, diffuse infiltration of medulla.

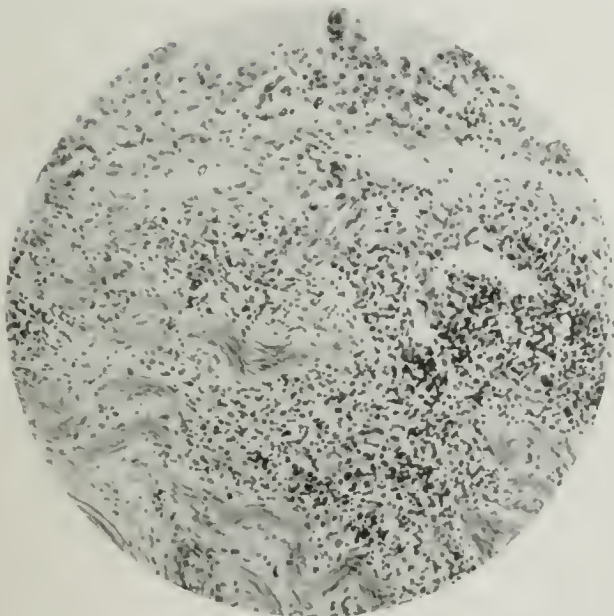


Fig. 13.
Rectum, ulceration and infiltration.

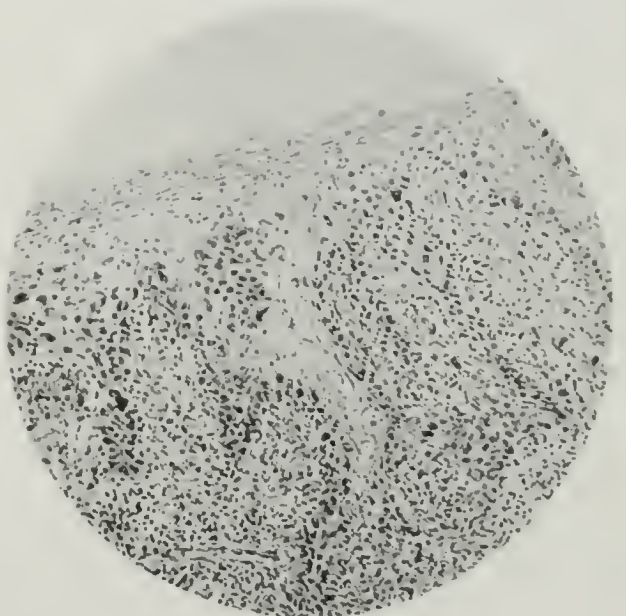


Fig. 14.
Tongue, active, subacute and chronic glossitis.

of the mucosa of the large bowel. Throughout the gastro-intestinal tract the nerves present a rather striking appearance. The cells of the endoneurium appear swollen and more bluish than normal. They are enlarged and oat-seed shaped as though they were edematous. The nerve fibers are unusually prominent. Whether this is due to the swelling of the fibers or to a possible atrophy of the muscularis might be debated. The writer does not believe that the muscularis is especially atrophic.

Near the anus is a rather large area of ulceration which microscopically presents an active infiltrative inflammatory picture (Plate IV, Fig. 13). The mucosa is eroded, the epithelium being replaced by granulation tissue actively infiltrated by round cells, which extend down to the deeper layers of the muscularis. There is here, then, a deeply destructive ulcerative process of the anus. There is not the marked atrophy of the muscularis in this case that most writers describe although the gross impression of muscular thinning was much greater than the microscopical appearances warranted.

Tongue.—The tongue (Plate IV, Fig. 14) presents a very striking appearance. There is marked atrophy of the epithelium with fraying out of the upper layers of the epithelium suggesting an atypical horny layer. The subepithelial tissues present a picture of extreme congestion and intense round-celled infiltration; a marked subacute but active glossitis.

Pancreas.—The pancreas presents no abnormal appearances either of the acini, ducts, islands or Langerhans, connective tissue, or blood-vessels.

Liver.—The liver shows chronic passive congestion with dilatation of the capillaries and corresponding atrophy of all the liver cells.

This picture is rather interesting, in that the blood is not collected especially around the central vein with atrophy and necrosis of the central cells as is usually the case, but the engorgement is universal throughout the liver, and the atrophy is diffuse and uniform throughout the lobule. This suggests a primary liver atrophy rather than one due to chronic passive congestion. Occasionally we come upon small focalized areas of round-celled infiltration, including plasma cells and occasional eosinophiles not in association with the islands of Glisson, but rather with the central or sublobular veins. Contrary to the impression obtained from gross inspection, no fat is found. The individual liver cells are swollen and edematous.

The *striped muscle* presents postmortem coagulation changes resembling Zenker's necrosis.

Genitourinary tract.—The cervix shows old ectropion and partially healed erosion, with chronic endocervicitis. Large cystic glands containing a large amount of mucous are present. Just inside the external os there is an area of polypoid granulation tissue extending down to the mouths of the glands and forming polypi on the surface.

The bladder mucosa is normal, except that one section shows a small area of round-celled infiltration around one of the vessels, and a very small area of round-celled infiltration in the mucosa in one place.

The kidney shows marked atrophy. There are many more glomeruli in a field than normal. There is no increase of connective tissue, either diffusely or around the vessels, and no vascular sclerosis. The tubules show no changes and

PLATE V.

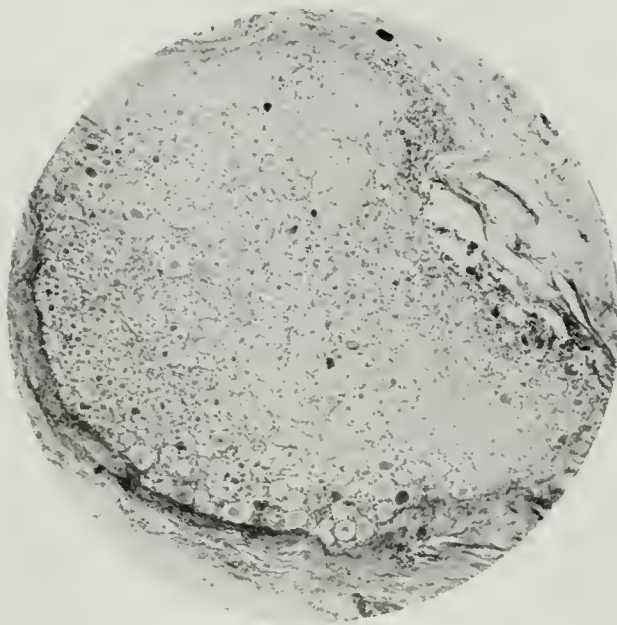


Fig. 15.
Posterior spinal ganglion, low power, showing degeneration.

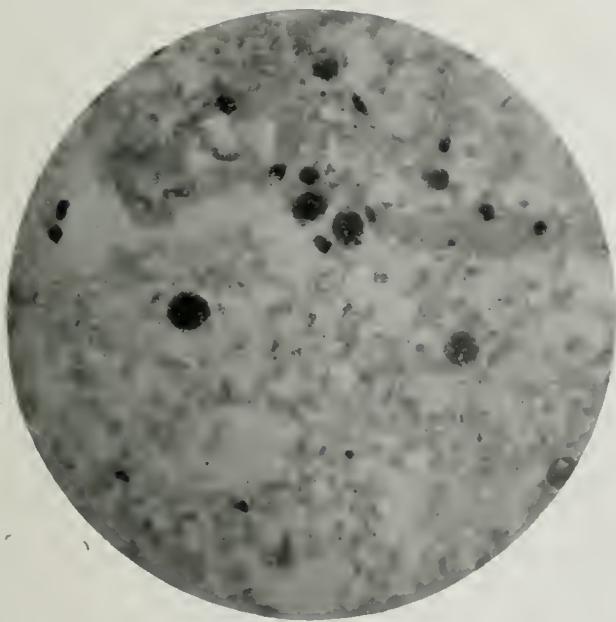


Fig. 16.
Calcified myelin droplets (?) in spinal cord.

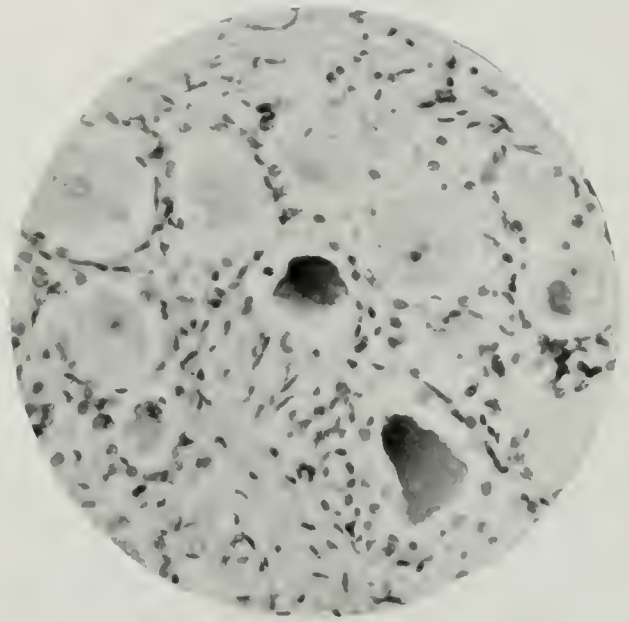


Fig. 17.
Cells of spinal ganglion, showing degeneration
of individual cells.

there is no round-celled infiltration. The only lesion presented by the kidney is one of extreme atrophy coupled with edema and congestion. The nephritic changes so commonly observed are lacking.

The ovary is atrophic. There is no general increase in connective tissue although there are a large number of corpora albicantia. The ovary is markedly congested. No active Graafian follicles are found. Both ovaries are the same size and neither is cystic. The tubes are normal, except for dilated varicose veins on their peritoneal surface. The uterine endometrium shows an extreme grade of atrophy although the vessels show less sclerosis than is usual with this extreme grade of atrophy of the endometrium.

Lung.—The lung shows moderate atrophy and anthracosis. There is an area of perivascular round-celled infiltration in the neighborhood of a large bronchus, near a small island of cartilage.

Central Nervous System.—The posterior root ganglia (Plate V, Figs. 15, 16, 17) present marked degeneration of individual cells, with marked shrinking and nuclear degeneration. There is striking brownish pigmentation of the ganglion cells as described by Lombroso and others. The spinal cord shows no sclerosis of any one particular tract but there is a general thinning of the myelin sheaths and numerous dark blue staining bodies (hematoxylin) from 20 to 50 microns in diameter distributed along the subpial region of the posterior columns and along the intramedullary portion of the posterior roots. The writer believes that many of the appearances described as degenerations of nerve cells are post-mortem changes and does not feel capable of reporting upon the nerve cell changes found in this case until he has had opportunity for further study and comparison. These bluish bodies have been observed in many pathological conditions, and may also be postmortem changes. A more complete report on the histopathology of the nervous system in this case will be made in the near future.

Skin.—The skin presents several important changes. There is marked hyperkeratinization of the epidermis everywhere (Plate II). The stratum Malpighii is markedly atrophic and in places the rete has dipped down deeply into the corium (Plate II, Fig. 6). The corium shows dilated fibroid vessels and increased amount of fibrous tissue with decrease in elastic tissue. The hair follicles are atrophic and deformed and are surrounded by rather thick sheaths of hyalin connective tissue (Plate II, Fig. 4). The sebaceous glands are almost absent and only fibroid remnants of them are to be found around the base of the hair follicles. The sweat glands are fairly normal, perhaps show a slight fibrosis. Here and there we find throughout the stratum corium areas of round-celled infiltration (Plate II, Fig. 4), usually perivascular but not always. This Gurd interprets as a healing reaction to the injury caused by light on the sensitized skin.

Spleen.—There is the usual picture of atrophy. The sinuses are engorged with blood. No evidence of increased blood destruction as reported by some observers is seen, either as evidenced by phagocytosis of red cells or increased pigmentation.

Bone-marrow.—The red marrow is increased in quantity and very active throughout all the bones. Microscopically the sections of the marrow are normal except for a general hyperplasia as is seen in any moderately severe anemia.

PLATE VI.

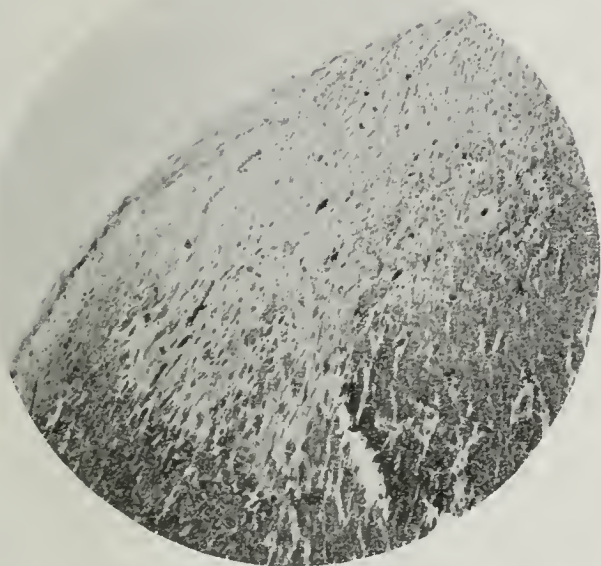


Fig. 18.
Anemic infarct of heart.

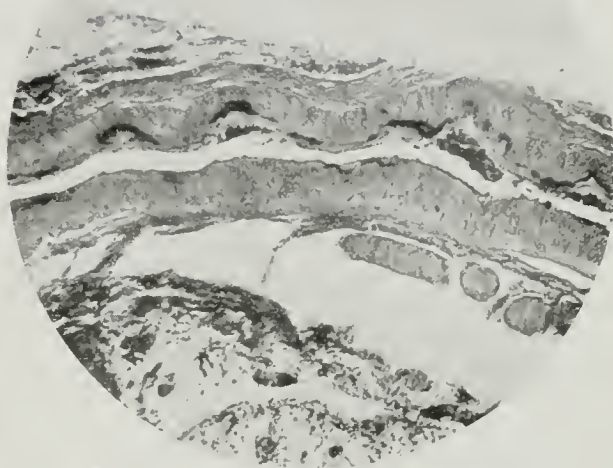


Fig. 19.
Sclerosis of coronary.

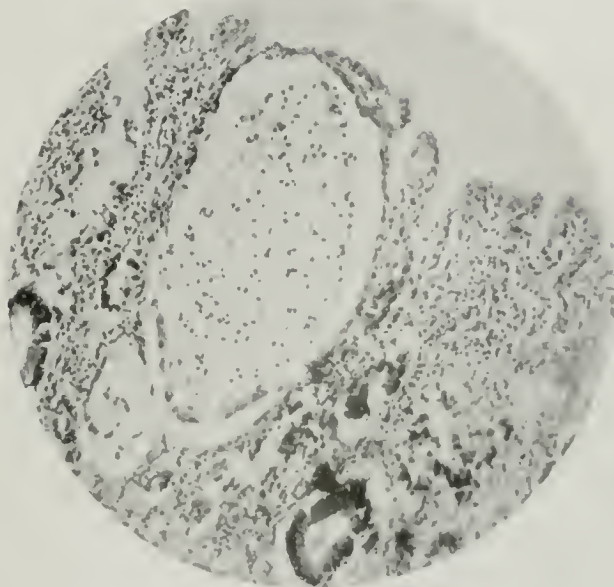


Fig. 20.
Large intestine, showing postmortem change and cystic degeneration of a gland.

SUMMARY OF POSTMORTEM FINDINGS.

Simple or brown atrophy of the heart, liver, spleen, lungs, intestinal muscles (?) and kidneys.

Degenerations and brownish pigmentation of the posterior root ganglia (and other groups of nerve cells).

Active ulcerative proctitis and chronic glossitis of marked degree.

Hyperkeratosis and sclerosis of the skin with areas of round-celled infiltration.

Hitherto undescribed changes of inflammatory nature in the thyroid and adrenal. The lesion in the thyroid being a marked example of chronic productive interstitial thyroiditis with compensatory reaction on the part of the thyroid follicles.

Small areas of round-celled infiltration in the thyroid, adrenal, liver, lung, bladder, tongue, rectum and skin.

Reviewing the findings in this case we find that the usual lesions observed in pellagra have been found here with the addition of striking changes observed in the thyroid and adrenals.

As far as the writer is able to determine, no such changes have been found in pellagrins. These changes in themselves would not be especially important if it could be found that careful microscopical examination of these organs had been frequently made in pellagrous individuals. The lesions take on added significance when we review the symptoms and signs of the disease. Even from our as yet incomplete and vague knowledge of the functions and relations of the ductless glands, we do not find it hard to reason on the basis of observed facts in other conditions, that such a syndrome as mental disturbance, cutaneous sensitization to injury by light or other injury, and gastrointestinal disturbance, might very well be secondary to functional disturbance in the thyroid and adrenal. Whether or not other autopsies made on the bodies of pellagrins will show these findings to be of constant occurrence or merely concomitant accidents due to some other primary cause, is a matter for further experience to decide.

There have been clinical reports of cases of pellagra in which the symptoms and signs suggested disease of the thyroid, adrenal, or parathyroid. Beeson reports a case giving evidence of thyroid disease, the signs of which were worse during the attacks of pellagra. Bertelli has recorded a case showing an Addisonian syndrome judging from the title of the paper, the original not being available to the writer. Paravicini has recorded a case of paralysis agitans associated with pellagra. This communication is also not available to me and has been read by title only.

An interesting finding recorded by several writers but especially reported lately by Hillman and Schulle is the lymphocytosis. This finding becomes doubly interesting in view of the thyroid changes found in this instance, since the occurrence of lymphocytosis in hyperthyroidism is well known.

The present case of course throws no direct light upon the question of whether pellagra is an infection of some form of toxic disturbance.

The impression which one gets from a careful perusal of the previous writ-

ings on the pathology of pellagra is that it requires quite a stretch of the imagination to reconcile the findings with an infectious disease. On the other hand, when we remember the psychoses of toxic origin, the gastrointestinal disturbances of toxic origin (either from vagus neuritis or from an effort on the part of the gastrointestinal tract to excrete the poison) and the toxic dermatoses, we find no a priori difficulty in reconciling the disease complex which we call pellagra with a toxic cause. In the light of the present findings we could imagine that this toxic state primarily injured the thyroid and adrenal and that some of the signs and symptoms of the disease as we know it are the secondary results of deranged gland function. It remains of course for further study to determine these points. One thing seems to the writer to be significant; namely, that the changes found in the thyroid and adrenal are unusual ones, thus lending support to the tendency to consider them of importance in the production of the clinical state presented by the patient.

I believe that the nervous lesions hitherto described and partially confirmed by my findings are wholly of toxic or nutritional origin. Many of the lesions to which much space has been given are surely postmortem changes and other artefacts, along with secondary changes which are common in many other conditions. The writer believes that reports of degenerations based upon the appearance of granules and other slight changes in nerve-cells have to be interpreted with extreme caution, inasmuch as so much depends upon the functional state of the cells at the time of death, postmortem change, fixative, method of preparation, etc. One of the safest criteria for the establishment of the fact of nerve cell degeneration is fat, none of which was demonstrable in my case. The nervous findings are being worked up separately with these ideas in mind.

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THE ANTAGONISTIC ACTION OF THE VAGUS AND SYMPATHETIC DIVISIONS OF THE AUTONOMIC NERVOUS SYSTEM IN PULMONARY TUBERCULOSIS.

BY FRANCIS M. POTTENGER, M.D., MONROVIA, CAL.

FOR a number of years I have interested myself in the study of reflex action as it affects the individual suffering from inflammation of the pulmonary parenchyma, particularly tuberculosis. This study has been difficult because of the fact that its very foundation is enveloped in mystery. In offering an explanation of the various phenomena noted, we are compelled to reason from effect to cause in a great many instances, because physiology has not yet made the paths of all the reflexes clear.

This study lies largely within the field of the autonomic nervous system, a field which is particularly characterized by our lack of knowledge, but one which offers great encouragement for future investigations.

The tuberculous patient cannot be understood clinically if we look upon him only as one afflicted with an infection of the pulmonary parenchyma, no matter how small the lesion. We must endeavor to know how the inflammation in the lung affects other organs which are supplied by the same system of nerves or which stand in such a relationship that they may be influenced reflexly; and we must study him as a human being suffering from a chronic disease which produces a chronic toxemia and one which is accompanied by a varied emotional state, both of which conditions exert a powerful influence upon the sympathetic nervous system. Realizing fully the difficulty of the task before me I shall endeavor to suggest some of the symptoms and conditions which arise from the irritation of the two great divisions of the autonomic system, the vagus and the sympathetic, and show the antagonistic action which is constantly going on.

I have been greatly stimulated in this study by the splendid work of Epinger and Hesz on Vagotonia ("Die Vagotonie," Sammlung. Klinischer Abhandlungen, von Noorden. Heft 9 u. 10, 1910) which has recently come into my hands, and those valuable and suggestive monographs by Cannon ("Bodily Changes in Pain, Hunger, Fear and Rage," Appleton, 1915) on the effect of emotions upon the human organism, and the studies of Beidl ("Innere Sekretion," Urban and Schwarzenberg, Wien, 1910) on the internal secretions.

The autonomic nervous system supplies impulses to structures which are not controlled by the will. These are the organs which are supplied by smooth muscles, such as the stomach, intestines, blood vessels, ducts of glands, skin and secreting glands; also certain organs possessing striated muscle fibres, such as the heart, the beginning and terminal portions of the alimentary canal, and the generative organs.

This system is characterized by the fact that under no circumstances do organs or parts receive innervation directly from a neuron whose cell bodies lie in the brain or spinal cord. Ganglia are interposed between the nerve cells in the central nervous system and the part innervated which act most prob-

ably by modifying impulses. In these ganglia are cell bodies belonging to neurons which have postganglionic fibres which go to supply the viscera.

The cerebrospinal nervous system, on the other hand, is controlled by the will. Its action is quick and definite. There are no ganglia interposed along the path of the nerve to modify the impulses which originate in the brain, but they are carried directly from cell bodies in the brain and cord to the muscles involved, and immediate action results.

The autonomic system consists of three divisions, the cranial, the thoracolumbar or sympathetic, and the sacral, as shown in Fig. 1 taken from Cannon. No connecting neurons for the autonomic system are given off from those portions of the cord which send out nerves to the fore and hind limbs.

Some writers divide the autonomic system into two divisions, and classify the cranial, bulbar and sacral divisions together as the vagus system, and the thoracic and upper lumbar as the sympathetic system.

The fibres of the sympathetic all pass through the ganglionated cord, while those which come from the cranial, bulbar and sacral portions of the cord do not.

The cranial fibres pass for the most part within the trunk of the nervus oculomotorius (III cranial), are interrupted in the ciliary ganglion, whence they furnish constrictor impulses to the sphincter of the iris.

The bulbar portion passes through the nervus facialis (VII cranial) and nervus glossopharyngeus (IX cranial) to the salivary glands and blood vessels of the mouth. Stimulation causes increased salivary flow in the one case and constriction of the vessels of the mouth in the other.

The most important branch of the autonomic coming from the bulbar portion of the cord is the nervus vagus (X cranial) which is the chief source of nerve supply for the internal viscera. It supplies the heart, bronchial tree, esophagus, stomach, intestines, pancreas, and liver.

The sacral branch is the nervus pelvicius. It innervates the descending colon, sigmoid, anus, bladder and generative organs.

The sympathetic fibres pass from the spinal canal in the thoracic and upper lumbar regions. They pass out and form the ganglionated cord and from this are widely distributed throughout the entire body. Many structures are innervated by both the vagus and the sympathetic systems, and wherever this double innervation is found, the action of the two is antagonistic; as in the eye, where the vagus contracts the pupil, the sympathetic dilates it; or in the heart—where the vagus slows, the sympathetic accelerates.

The cranial and sacral divisions of the autonomic system have a more direct action than the sympathetic because the fibres coming from the cranial and sacral divisions run more directly to the organs innervated than do those of the sympathetic. Thus, the fibres of the vagus supplying the heart and gastrointestinal tract do not enter ganglia until they reach the substance of the organs, while the sympathetic fibres pass through several ganglia each of which complicates and modifies their action. Consequently, stimulation of the cranial, bulbar, and sacral fibres of the autonomic system may cause a resultant action in some individual organ, while stimulation of the sympathetic fibres causes a widespread effect involving several or many organs. This affords us a physio-

logical explanation for the general widespread character of sympathetic impulses.

The following table shows the antagonistic action between the vagus and sympathetic systems in the principal structures concerned in our study:

ACTION OF THE SYMPATHETICS WHEN STIMULATED.	ORGAN	ACTION OF THE AUTONOMIC WHEN STIMULATED.
I-II thoracic	dilates pupil contracts pupil	III N.
I-III thoracic	stimulates orbital muscle stimulates m. ciliaris	III N.
II-IV thoracic	inhibits salivary secretion stimulates salivary secretion.....	Chorda tympani
II-IV thoracic	contracts vessels of skin of head and vessels of brain dilates vessels of brain.....	X N. IX N.
*II Th.-IV L..... I-IV lumbar	contracts intestinal vessels contracts genital vessels dilates genital vessels.....	N. pelvici
II Th.-IV L..... IV-VII Th.	stimulates sweat glands stimulates hair muscles of face and head	
I-V Th.	accelerates heart contractions slows heart contractions.....	N. X
II-IV Th.	relaxes oesophagus contracts oesophagus	N. X
II Th.-IV L.	relaxes cardia contracts cardia	N. X
II Th.-IV L.	diminishes gastric tonus increases gastric tonus.....	N. X
II Th.-IV L.	inhibits gastric peristalsis increases gastric peristalsis.....	N. X
II Th.-IV L.	diminishes gastric secretion increases gastric secretion	N. X
II Th.-IV L.	inhibits motility of small intestine stimulates motility of small intestine....	N. X
II Th.-IV L.	inhibits (?) pancreas secretion stimulates pancreas secretion.....	N. X
I-IV L.	relaxes contraction of colon stimulates contraction of colon.....	N. pelvici
I-IV L.	relaxes sphincter ani stimulates sphincter ani.....	N. pelvici N. X
II Th.-IV L.	contracts and dilates vessels of upper and lower extremities.	

*Th.—thoracic. L. Lumbar.

The vagus system takes part in many reflex acts. The sympathetic also carries afferent impulses, when its end fibres in any organ are irritated, which result in reflex action; and, besides, the system as a whole, is influenced greatly by toxic conditions and by emotional states such as fear, anxiety, discouragement and disappointment.

In pulmonary tuberculosis it can readily be seen that we have a most complex study. There is primarily an inflammation of tissue which irritates filaments belonging to one of the chief branches of the vagus system. Resulting from this, afferent impulses are sent to the cell bodies in the central nervous system and are there transmitted to other cells and through them to fibres which pass out through other branches of the vagus system to produce increased vagus tone in the fibres affected. This increased vagus tone may be distributed more or less generally through the different branches of the vagus system or it may be selective in that it affects certain branches more than others.

Along with the vagus stimulation resulting in increase in vagus tonus, there is a stimulation of the sympathetics, which results in a reflex increase in sympathetic tonus in practically the same organs. Whether sympathetic or vagus tonus shall predominate depends on the conditions present. Cannon has demonstrated the effect of fear, rage and pain on the organism. In tuberculosis we have a central stimulation of sympathetics by the toxins and an exaggerated emotional state, as a result of the disease which also acts as a stimulant upon the sympathetics. Allied to fear and rage are malaise and depressive nervous states which accompany the tuberculous toxemia and which, acting through the sympathetics, have a general antagonistic action to vagus impulses wherever met.

In this connection it is interesting to note how closely the emotional states and toxemias (in this case tuberculous toxemia) are allied in their symptomatology and end effects. The individual in a state of great emotion, shows dilatation of the pupil, pallor, feels his hairs rise on end, suffers from dryness of the mouth, rapid pulse, a stoppage of digestion and other similar symptoms which are characteristic of sympathetic irritation. The toxemia which accompanies tuberculosis, the same as all other toxemias, produces general sympathetic stimulation. The nervous condition resulting is characterized by malaise, anxiety, discouragement, fear and general depression; and these, in turn, cause the same chain of symptoms, only not so pronounced, as a rule, as those which follow violent emotion. Such patients, however, during the periods of marked toxemia, suffer from pallor, rapid pulse, dry mouth, coated tongue, disturbed appetite, slowness of digestion and stasis of intestinal contents. During slight toxemia these symptoms may not be noticed or they may be very slight in character. The symptom-complex of toxemia is an expression of general discharge through the sympathetic nervous system, and is practically the same, though different in degree, no matter what the nature of the toxemia.

The action of the sympathetic on the sacral division is also evident in toxemia as shown in the inhibition of the sexual desire. It is not at all impossible that the increased desire which so often marks periods of improvement in tuberculosis is evidence of increased sacral tonus, and an overbalancing of the equilibrium normally maintained between the sacral and the sympathetic. Inasmuch as these changes in this division of the autonomic system are not so im-

portant in symptomatology as those in the vagus system we shall not discuss them further at this time.

The above symptoms are not constantly present. Variability characterizes the symptoms produced by nervous stimulation in early tuberculosis. This is due to the fact that several important and antagonistic influences are operative at the same time; and also to the fact that the tuberculous inflammation in the lung goes through cycles of greater and lesser activity, producing varying degrees of stimulation; and, further, to the fact that the relative vagus and sympathetic tonus differs in different individuals.

The nerve endings of both the vagus and sympathetic systems are irritated by the pulmonary inflammation; and through their respective fibres afferent impulses are sent centralwards, which result in reflex stimulation of other branches of their respective systems or of nerves with which they communicate, going out to supply such other organs as the eye, heart and gastrointestinal tract. Sometimes the stimulation is greater in the vagus system; at other times in the sympathetic. As a rule, however, except during the periods of increased toxemia, which I shall discuss later, my observation leads me to believe that the vagus tonus is greater than the sympathetic tonus in the majority of patients suffering from early tuberculosis. This seems to be true in nearly all except those of the distinctly asthenic type. This is shown on the part of the heart and particularly on the part of the gastrointestinal tract, as described later.

In estimating the relative tonus of the vagus and sympathetic systems it is necessary to bear in mind that increased tonus might show in one division of nerves and not in all; and that this increased tonus in particular divisions might not be constant. This variability is due to the fact that there are many factors causing the stimulation and that some of these have selective action for certain structures. This is not only evident in the reflexes, but also in the internal secretions which affect the two systems. It is also evident from the fact that the tuberculous patient may have increased or decreased tonus in either the vagus or sympathetic systems from causes other than those which operate as a result of the pulmonary infection. From the antagonistic action of the vagus and sympathetic systems the function of many organs is rendered unstable. The heart particularly in early and nonactive tuberculosis while at rest, is often slower than normal; but on exertion or during periods of emotion at once becomes more rapid than normal and settles down to the normal much slower than it should. The appetite, while disturbed during acute toxic states and during conditions which favor increased absorption of toxins, such as overexertion, or during periods of anxiety and discouragement, is often normal and even sharper than normal when the patient is put at rest in the open air under favorable circumstances which offer him hope of cure. The same is true of the gastric and intestinal disturbances. Hyperacidity and hypermotility are the rule in early tuberculosis, when toxemia is relieved. The intestinal tract also shows the same increased tonus. The vagus system, the one which conserves the healthful action of the important internal organs, overcomes its antagonist in early tuberculosis for the most of the time and thus offers the patient an increased opportunity for restoration to health.

During the waves of increased activity in the tuberculous focus, however,

and when the patient is causing increased absorption of toxins by overexertion and other faulty habits, likewise during periods of discouragement and mental depression, which are not uncommon, the excessive stimulation of the sympathetic system produced by both reflex action, and the toxemia and emotional states, overcomes the increased vagus tonus and causes symptoms which are characteristic of sympathetic irritation, such as rapid heart, decreased appetite, hypochlorhydria, deficient gastric motility and alteration in the secretion and motility of the intestinal tract. Whether this is wholly a direct action upon the sympathetic system or partly a general disturbance of internal secretions is not clear, but the effect is that of a general discharge through the sympathetic system.

The extent to which the sympathetic tonus can be relieved is a factor in prognosis. As the disease increases in virulence and activity, the vagus tonus gradually yields to the supremacy of the sympathetic.

Aside from the antagonistic action of these two systems it seems well established that antagonistic fibres are at times supplied to organs by the same system.

In health a state of equilibrium is maintained in the various organs as a result of the antagonistic action of these two systems. In any disease which affects either by stimulation or by setting aside the normal nerve tonus of any important branch or group of fibres of either or both of these systems, there is a consequent disturbance of equilibrium which results in a pathological state.

This is evident in the production of nausea through eye strain, or the slowing of the heart in abdominal lesions which affect the depressor fibres of the vagus. So it is evident in pulmonary inflammation.

Aside from the direct stimulation of the nerves by inflammatory processes we must recognize the influence of substances of a chemical nature which act either upon terminal filaments or nerve centers. The primary action of the toxins is a direct stimulation. The secondary action may be a totally different one and depend on certain secretions (hormones) which are set free as a result of the primary toxic influence, such as occurs when adrenalin is set free by certain emotional states or gastric secretion is stimulated by the smell or taste of savory food.

It is more and more believed as we study deeper into the problems of physiology, that the harmonious and dysharmonious action of cells and special groups of cells is increased not only by direct nerve stimulation, but also by internal secretions which, coming from some distant gland, act as hormones or messengers calling forth normal harmonious action when the parts are in a normal state, and abnormal action when the parts are abnormal. Such action is on the organ itself and not on the nerve supplying it.

This is evident in case of the thyroid, suprarenals, pituitary body, testicles and ovary; and probably just as true in the case of such other organs as the kidney, liver, spleen, pancreas and even such secreting glands as those in the gastrointestinal tract. A dysfunction of one gland may be followed by a disturbance in others because of a failure of the normal secretion of that gland to course in the blood and produce its relative stimulation of other glands. The stimulation of these glands which results in the production of such hormones is at least partially under direct nerve control; and, in this manner, the stim-

ulation of a nerve supplying a given organ may not only affect that particular organ, but one widely separated from it through the internal secretion which is affected by such stimulation.

No doubt future study will clarify this subject very much by the discovery of the true relationship which exists between many of the internal secretions and the structures controlled by them. An important beginning has been made in this direction in the discovery of the fact that the secretion of adrenin is stimulated by the same conditions (emotional states) which stimulate the sympathetic nervous system (Cannon), and that when this substance is thrown out into the circulation, it finds its way to the various structures controlled by the sympathetic nervous system and exerts for the most part, the same antagonistic action against the vagus as the sympathetic itself. This may partly explain the action of toxemia as well as a central stimulation of the sympathetic neurons. There are doubtless other organs which produce hormones antagonistic to sympathetic action; in fact, the observation that dogs whose pancreas are removed have an overstimulation of the adrenal system indicates that such a hormone is produced in the pancreas.

Let us now proceed to apply these principles to pulmonary tuberculosis and the explanation of such phenomena as are caused by the disturbed balance in the vagus and sympathetic systems.

In tuberculosis we must conceive of a condition in which both the vagus and sympathetic systems are simultaneously stimulated, but one in which either one or the other usually yields with a resultant disturbed equilibrium.

Pupil. At least fifty per cent of tuberculous patients show a dilated pupil on the side of involvement during the time that the inflammation is acute; and it is probable that a much larger per cent would show it if observation could be made continuously. This indicates that the action of the sympathetic overcomes that of the vagus. This dilator effect is due to irritation of the fibres from the Ist and IIInd thoracic segments of the cord. Artificial stimulation of these fibres causes the same dilator effect. Aside from the pupillary effect, the equilibrium of the ciliary body and orbital muscle is disturbed. I am led to believe, that, aside from the dilatation of the pupil, there is a serious disturbance in the muscle balance resulting from the antagonistic action of the vagus and sympathetic which causes a disturbance of accommodation. It is surprising to see how many patients complain of headache, if they continue to use their eyes for reading, knitting, or sewing, as they have been accustomed to do prior to their illness. They also seem to show an increased and abnormal sensitiveness to bright light; and I often find it necessary to suggest that the bed be so placed that the light will not shine directly in the eyes. Change of eye glasses is made necessary more often than prior to illness even in early and chronic cases which are only slightly active. This is usually explained as being due to the lowered physical state; but may not this altered muscle balance be the cause?

Hectic flush. The vasoconstrictor fibres of the cervical sympathetics take their origin from the upper thoracic roots, particularly from the IIId, IIIId and IVth, and supply the vessels of the face and head. Hectic flush is present, for the most part, only during periods of acute toxemia, and comes on only after a considerable amount of tissue is involved. It is confined to the side affected,

the same as is noted in the reflex dilatations of the pupil. This of itself suggests that reflex action is at least the localizing factor, although the toxic action may also be required to overcome the constrictor effect.

Heart. In the heart, in tuberculosis, we have so many conditions present which influence the pulse rate that it is impossible to accurately ascribe to the vagus and sympathetic systems the part which each plays. Impulses are carried through both systems, because both are more or less constantly irritated.

As a result of this double source of impulses, the one through the vagus tending to slow the heart, the other through the sympathetic, attempting to quicken its action, there is a marked disturbance in equilibrium. This shows early in the disease before such conditions as loss of pulmonary tissue, heart strain, and degenerative changes can be considered. The heart beat may be normal, or only slightly faster than normal, while the patient is at rest; but unduly rapid on exertion. Rapidity is also increased during states of toxemia and during periods of depression.

This characteristic relative slowness of the heart beat while at rest is sometimes noticed even during periods of temperature in advanced tuberculosis. When compared with the pulse rate accompanying the same degree of temperature in other diseases it is often lower. This is most probably a result of an inhibitory reflex through the vagus, the impulse coming from the irritation in the lung. When the intestinal tract is affected with tuberculosis, then another division of the great vagus system is stimulated and we often see this inhibitory action accentuated, with a still greater departure in pulse rate from that which would be expected with the degree of temperature present. If an unusual slowing of the pulse occurs in the course of pulmonary tuberculosis coincident with an elevation of one or two degrees in the temperature curve, reflex vagus irritation should be considered as a probable cause and a complicating intestinal tuberculosis be suspected.

Irritation of other branches of the vagus will also cause inhibitory action upon the heart. Thus pressing upon the eyeball and irritating the nasal mucous membrane will both cause slowing of the heart; so does intracranial pressure as shown at times in tumors and conditions accompanied by fluid.

Opposing this inhibitory action through the vagus, we have acceleration through the sympathetic, either by direct stimulation, or through toxins or through the various emotional states which affect the patient. Often the sympathetic irritation gains the upper hand and a markedly rapid pulse results.

Gastrointestinal tract. The antagonistic action of the vagus and sympathetic on the different portions of the intestinal tract is extremely interesting; and our understanding of this action will aid greatly in forming an accurate clinical conception of the digestive capabilities of the tuberculous patient.

Early in tuberculosis the toxemia present is neither constant nor of a high degree as mentioned above, and its action upon the sympathetic is negligible as compared with that present in the more advanced cases. From the very first, however, vagus stimulation seems to be important. This is shown in the larynx, in hoarseness due to interference with the innervation, and also in the irritation which produces cough. It is likewise marked in the intestinal tract, as I shall now describe.

Increased salivary flow. This is present at times in tuberculous patients, though by no means constant. This could be due to reflex stimulation of the salivary glands through the vagus branch which courses with the chorda tympani. The dry mouth and coated tongue are almost always present during the periods of toxemia, and, at times, when this has passed away. This is an expression of increased sympathetic action.

Coated Tongue. A dry coating of the tongue is often noticed during periods of temperature in cases where toxemia is a factor. The drying effect on the secretions through the sympathetics must be thought of as an etiological factor. The fact that this is nearly always accompanied by a diminution of appetite and deficiency in gastric and intestinal efficiency makes the cause the more probable, for these other conditions are likewise due to deficient vagus tonus or increased sympathetic tonus.

Stomach. The disturbance on the part of the stomach in early tuberculosis, except during periods of acute toxemia and periods of depression are those which belong to the class of so-called nervous dyspepsias. The form which seems most common is a slight hyperacidity. This may be brought about by a reflex through the vagus, the impulse coming from the inflamed pulmonary parenchyma. The digestive powers are, if anything, above par. This may be a reason why the tuberculous patient at these times is able to care for such large amounts of food. Associated with the increased secretion there is also an increased motility and at times, a spastic constipation. This increased vagus tonus sometimes shows itself in a feeling which approaches nausea. These conditions, however, are not continuous. They alternate with decreased vagus tonus as mentioned above. This increased vagus tonus can be relieved by the administration of atropin, and following its administration hyperacidity and hypermotility often disappear. There are several factors which come in to cause sympathetic stimulation and inhibition of vagus action, notably reflex irritation, the toxemia and the depressed nervous state which is so common and which is characterized by mental depression, anxiety, discouragement and fear. These emotional states come and go all through the disease. They are sometimes dependent upon and sometimes independent of toxemia.

Intestines. A similar condition obtains in the intestinal tract where we have states of increased vagus tonus, causing increased secretion and increased motility, and abnormal sympathetic irritation inhibiting this and interfering with secretion and peristaltic action. Spastic constipation as a result of increased vagus tonus is common in early tuberculosis, while the atonic type is the rule later. Definite effects of each system are not so easy to point out in the intestinal tract because of the preponderance of stasis and constipation, which are regularly found in general life and which affect so many of those who are afflicted with tuberculosis prior to the time when the disease becomes manifest. We can say, however, that, as a rule, digestion becomes impaired and stasis and constipation become more pronounced as the disease advances and toxemia and the various depressive emotional states become more marked.

Thus, it would seem that early tuberculosis is largely a condition in which increased vagus tonus predominates over sympathetic stimulation, and advanced

tuberculosis a condition in which sympathetic irritation seems to be greater than the vagus tonus.

In offering this analysis of nervous action in tuberculosis, I realize fully that it is impossible to always point out the direct relationship between cause and effect; because we are dealing with a disease which produces dysfunction on the part of many organs and which results in a multitude of conditions which might produce symptoms similar to those which could be explained at one time by irritation of the sympathetics, at another by irritation of the vagus. It can be seen, however, that these two systems are to be taken into account, and that, as long as inflammation in the lung exists, so long are impulses carried to various organs through these two systems, which, acting antagonistically, disturb the normal equilibrium, which is so necessary to normal function.

ETIOLOGY AND LABORATORY DIAGNOSIS OF SMALLPOX AND CHICKENPOX*

BY J. N. FORCE, M.D., BERKELEY, CAL.

ELEVEN years after Jenner published his monograph on vaccination, Sacco recorded the presence of granules in the lymph contained in the vaccine vesicle, but it was not until 1868, however, that Chauveau¹ was able to show by diffusion experiments, that the active principle of vaccine virus was contained in these granules and not in the fluid in which they were suspended.

Beginning with Cohn² in 1872, a number of observers made careful studies of the bacteria found in the vaccine vesicle, but no one was able to produce the lesions of vaccinia by means of animal inoculation with a pure culture of any one of these bacteria.

De Waele and Sugg³ during a smallpox epidemic succeeded in isolating from the blood of persons dead of the disease, a streptococcus which was agglutinated by the serum of smallpox cases in dilutions of 1 in 800. The same organism was isolated from the vesicles of vaccinia cases and was agglutinated by the blood of other vaccinia cases. These same observers⁴ isolated a streptococcus from chickenpox vesicles which, though readily agglutinated by the serum of chickenpox cases, was not agglutinated by the serum of smallpox cases, vaccinia cases, or new-born infants. After a large number of control experiments with other streptococci, de Waele and Sugg announced the specificity of these streptococci and suggested the agglutination test as a means for the differential diagnosis of smallpox and chickenpox. It is indeed remarkable that so definite a statement involving both morphology and immunology of supposed causative agents for these two diseases should be neither confirmed nor denied by other observers. It is possible that the scientific world hesitated to recognize streptococcus variolæ until specific immunity had been produced in animals by inoculation with a pure culture of the newly discovered organism.

The failure to produce vaccinia with any bacteria contained in the vaccine vesicle naturally suggested that these bacteria were in the nature of secondary

*From the Department of Hygiene, University of California.

invaders and led to a desire to see how the vaccine lymph would behave if free from this contamination. Cheyne⁵ in 1850 suggested the use of vaccine virus mixed with glycerin as a substitute for the dry point, though this suggestion was made in the interest of convenience of handling and preservation rather than removing contamination. Copeman⁶ in 1891 showed that vaccine virus mixed with fifty per cent glycerin would retain its activity even though the bacterial content of the mixture gradually lessened. The following year Chambon, Menard, and Straus⁷ came to the same conclusion independently since they were able to produce typical vaccinia lesions with a mixture of virus and glycerin sixty days old. The mixture was entirely free from the usual bacteria as was shown by plating experiments.

The fact that typical vaccine vesicles could be produced by a bacteria free virus suggested a protozoan origin for the disease. Guarnieri⁸ in 1892 gave the name cytoryctes to certain bodies which he found constantly occurring in the epithelial cells of smallpox vesicles as well as in the corneal epithelial cells of rabbits fifty hours after inoculation of the cornea with vaccine virus. These bodies were extranuclear in the epithelial cells and varied in shape and size, being on the average half the size of the cell nucleus. Councilman, Brinckerhoff, and Tyzzer⁹ believed that the bodies or cytoryctes of Guarnieri were the true parasites of smallpox and vaccinia. They also described a second form which was present within the nucleus of the epithelial cell in smallpox only. They considered the forms to represent two stages in the life history of the same organism and ascribed the differences between variola inoculata and vaccinia in monkeys to the presence of the intranuclear form in the former disease. The intranuclear forms were most abundant in the vesicles of a general eruption produced by the intravenous inoculation of smallpox virus, but were only occasionally found in the lesions produced by skin inoculation of the same virus. The extranuclear forms or original cytoryctes were present in both smallpox and vaccinia lesions.

In connection with the above studies, Tyzzer¹⁰ carried on an independent investigation of the histology of the chickenpox vesicle. Though unable to produce any reaction in the corneal epithelial cells of a rabbit or in the skin of a monkey by inoculation with chickenpox vesicle contents, he described certain bodies enclosed in the nuclei and cytoplasm of the epithelial cells of the chickenpox vesicle. Associated with appearance of these bodies is a division of the nucleus and an enlargement of the epithelial cells. The resulting large polynuclear cells, Tyzzer considered characteristic of chickenpox. He did not consider the inclusion bodies parasitic in character nor did he think that they would be confused with cytoryctes by an experienced observer. The inability of Tyzzer to produce lesions with chickenpox material in the corneal cells of rabbits was in the nature of a confirmation of the observations of Salmon¹¹ who was unable to affect the cornea with either chickenpox or confluent acne material, though finding it easily affected by both smallpox and vaccine virus.

The newly discovered cytoryctes were not universally accepted as the causative agent of smallpox. In the year following the publication of Guarnieri's discovery we find Ferroni and Massai¹² expressing a belief that Guarnieri's parasites were irritation bodies derived from the nuclei of the epithelial cells.

This belief was based on corneal inoculations with croton oil and india ink. Resulting stained sections showed bodies practically identical with the cytoryctes. Further confirmation of the nonparasitic character of these bodies was offered by Salmon¹³ who showed that the staining reactions of the parasites, while differing from those of the nucleus of the epithelial cell, corresponded to those of the nuclei of migrating leucocytes whose chromatin masses tend to break down in the invaded cells. Foa¹⁴ also denied the parasitic nature of the cytoryctes on the ground of a nonprotozoan morphology, the resistance of vaccine virus to physical and chemical agents capable of destroying the cytoryctes, and finally that analogous forms found in sheep-pox will not traverse the filter which allows passage of the sheep-pox virus. Von Prowazek¹⁵ discovered that the young cytoryctes were tied by filaments to the cell nucleus. He did not consider them parasites, products of degeneration, or even the nuclei of wandering leucocytes, but rather a hyperproduction of nuclear material on the part of the epithelial cell as a reaction to stimulation produced by the smallpox organism. He believed that the extruded nuclear material surrounded the organism and suggested the group name chlamydozoa (chlamys, a cloak) for all organisms causing similar reactions. He admitted that bodies resembling cytoryctes could be produced by the irritation of the cornea with various substances, e. g., trypsin, but held that the production of true cytoryctes was a specific property of the smallpox organism. Finally he showed that cytoryctes could not be produced in the corneal cells of rabbits by inoculation with heated vaccine virus, though the same heated virus would produce immunity if injected subcutaneously.

It has been stated above that Tyzzer noted inclusion bodies in the epithelial cells of the chickenpox vesicle. He did not consider these parasitic, was unable to produce them in corneal lesions, and did not consider them readily confused with the cytoryctes. On the other hand Keysselitz and Mayer¹⁶ believed the chickenpox inclusion bodies to be analogous to the cytoryctes though they believed that the actual organism of chickenpox is located within the inclusion bodies, thus following the chlamydozoa theory of von Prowazek. Bertarelli¹⁷ was unable to inoculate either child, monkey, dog, or guinea-pig with chickenpox vesicle contents or dried crusts. He did, however, in contradistinction to Tyzzer, succeed in producing a corneal reaction in rabbits which was characterized by inclusion bodies in the epithelial cells. The bodies were not as large or as deeply stained as the cytoryctes, but they were not confusable with the irritation forms produced by osmic acid or bacterial toxins. Swellengrebel¹⁸ confirmed the observation of Bertarelli concerning the staining characters of the chickenpox inclusion bodies. He differentiated them from the cytoryctes by a structure more like the nucleus in character, and a location more often within the nucleus of the epithelial cell, though these differences were not at all constant.

Aside from the corneal inoculations with chickenpox vesicle contents, the only results of inoculation which seem to be beyond question are those of Kling.¹⁹ Kling easily inoculated healthy children by pricking the skin of the arm with a lancet charged with fluid from a fresh chickenpox vesicle. At the end of eight days, a typical chickenpox vesicle appeared, which was transmitted for five passages. The vesicle appeared in children recently vaccinated against small-

pox which shows that the lesion produced was not due to vaccinia. Out of thirty-one children inoculated in this manner during an epidemic in a children's hospital, only one had a febrile attack of chickenpox. In the same hospital there were thirty-two cases of chickenpox out of sixty-four unvaricellated children. Rabinoff³⁹ has recently applied the varicellation method of Kling to seventy-six children in an infant asylum. The six cases of chickenpox which appeared in this group all occurred within ten days after varicellation, i. e., within the incubation period of the disease. Out of one hundred and forty-two susceptible children in the same asylum, there were one hundred and fourteen cases of chickenpox. These figures represent an incidence of eight per cent in varicellated cases as against seventy-five per cent in susceptible cases.

These observations of Kling in conjunction with the findings of Bertarelli and Swellengrebel show that the clinical similarity of chickenpox and mild smallpox is confirmed by a similarity of the reaction of the epithelial cell to the invasion of either organism, as well as a similarity in the protection afforded by both vaccination and varicellation. On the other hand, the fact that a smallpox immune child could be varicellated indicates that the two diseases are not modifications of the same process as believed by Hebra.

It is needless to say that while the studies on cytoryctes were in progress, many attempts were made to pass the smallpox organism through the Berkefeld filter. All these experiments resulted in failure until Nicolle and Adil-Bey²⁰ in 1900 passed vaccine virus through the filter after a preliminary digestion of the vaccine lymph with pancreatic juice. Negri²¹ diluted fresh unglycerinated pulp with ten to twelve times its weight of water and ground it in a mortar. The ground pulp was allowed to stand on ice for several days and then reground in a Csoker grinding machine. The filtrate from the resulting emulsion gave typical corneal reactions including cytoryctes though a large quantity of the filtrate and a ten hour contact with the cornea were necessary. Green²² collected vaccine pulp, triturated it with saline solution, heated at 60° C. for one hour, and filtered through the Berkefeld filter. The filtrate injected subcutaneously into guinea-pigs, monkeys, and calves produced immunity against subsequent skin vaccination. In a later communication, Green²³ reports inability to produce any trace of vaccinia in calves and guinea-pigs vaccinated with the filtrate of vaccine pulp removed from calves ninety-six hours after vaccination and diluted 1 in 8 with five per cent sodium citrate solution. Foster and Force treated glycerinated vaccine virus with ether, removed the ether, diluted the vaccine virus 1 in 50 with saline solution, and centrifuged at high speed. The supernatant fluid was divided into two portions, one of which being passed through the Berkefeld filter. Guinea-pigs were then inoculated subcutaneously with equal doses of the filtered and unfiltered portions. Subsequent scrotal vaccination of the guinea-pigs produced abortive vesicles where the animals had been inoculated with the filtrate, and distinct allergic reactions where the animals had been vaccinated with the unfiltered supernatant fluid. Repeating the experiment with unetherized virus gave similar results. Control scrotal vaccination with the original virus gave typical vesicle formation.

Experiences in the filtration of vaccine virus indicate that the organism is contained in some type of surrounding substance and is incapable of passing the filter until this substance is removed by digestion, grinding, or the action

of solvents. This is strictly in accord with the chlamydozoa theory of von Prowazek. It is also evident that the activity of the virus is reduced by filtration, whether by mechanical action of the filter, or by inability of the organisms to separate in sufficient quantities from their surrounding cytocytes. This is shown by the partial protection of guinea-pigs receiving a subcutaneous inoculation of filtrate as contrasted with the complete protection afforded by an equal quantity of ground, centrifuged, but unfiltered vaccine virus. Whatever effect the ether may have had in dissolving the cytocytes was offset by the marked tendency of the etherized virus to clog the filter.

Von Prowazek gave the name chlamydozoa to organisms characterized by the formation of a surrounding cloak derived from the nucleus of the invaded cell. Fornet²⁴ suggested the name microsoma to designate the group of pathogenic germs to which belong the bodies described by Flexner and Noguchi in poliomyelitis and rabies, as well as the smallpox organisms. Microsoma variolae, according to Fornet, occur in pairs surrounded by a narrow halo which is easily seen when stained by Giemsa's solution. They are agglutinated completely by the serum of a smallpox case. Volpino²⁵ under the name of "corpuscles mobiles" described similar bodies seen with the ultramicroscope in the corneal epithelial cells of the rabbit. These bodies do not exceed 0.2 microns in diameter, are very numerous, and very motile. Volpino laid great stress on the diameter of these bodies as distinguished from those described by Paschen²⁶ which measured 0.5 microns and were contained in fresh lymph taken from a child. Volpino considered these to be leucocytic granules.

The most careful and comprehensive study of the morphology of the smallpox organism is that of Belin.²⁷ After vaccinating the cornea of a rabbit, he sutured the eyelids together assuming that the tears would rid the site of extraneous organisms and leave a practically pure culture of the smallpox organism. Studies made of the pus resulting from this vaccination showed the following forms against the dark field:

1. Motile coccoid forms less than 0.5 micron in diameter which were agglutinated by the serum of smallpox immunes.
2. Similar forms within the corneal cells.
3. A very few large oval bodies sometimes as large as 2.0 microns.
4. Spirochaetes occurring early, very fragile, and in many cases containing in one end a small body similar to the free coccoid form of the organism.

In a later communication, Belin²⁸ states that he was able to obtain all these forms from a serum broth to which had been added small particles of rabbit skin inoculated with vaccine virus. Belin claims that reproduction of the organism took place in this culture medium since vaccination on the backs of rabbits with emulsions made from skin particles fished from the broth produced confluent eruption on using particles which had been in the broth four days, where only a few discrete vesicles were produced by using particles which had been in the broth a shorter time. A portion of fourth-day emulsion produced discrete vesicles after being passed through a Berkefeld filter.

The work of Belin furnishes a combined study of morphology and attempts to cultivate the vaccine organism *in vitro*. Several other reports have been made recently on the cultivation of a bacteria free vaccine both *in vivo* and *in*

vitro. Steinhardt, Israeli, and Lambert²⁹ repeated the work of Belin using fragments of rabbit cornea inoculated with vaccine virus and incubated at 37° C. The incubated corneal fragments gave rise to confluent eruptions on the backs of rabbits, while unincubated controls gave only discrete vesicles. Steinhardt-Harde³⁰ secured a bacteria-free vaccine by the dialysis of commercial glycerinated virus through collodion sacks. This process removed the glycerin and phenol. The contents of the sacks were either plated on agar or re-dialysed with 0.5 per cent phenol. In the first instance the spaces between the contaminating organisms which appeared on the agar plates were carefully cut out and the vaccine removed therefrom. In the second instance the re-dialysis gave rise to a bacteria-free liquid virus which was much more active than when the bacteria had been killed by shaking with ether as advocated by Fernet. Fernet,²⁴ however, holds that shaking with ether does not seriously impair the vitality of the virus, since he succeeded in transplanting the organism for seven generations in serum broth to which a piece of sponge platinum had been added. This growth was secured after the removal of contaminating bacteria by means of ether. Fernet was able to vaccinate a calf with the seventh generation broth culture and is convinced that this result was not due to dilution of the original material, since this would represent a dilution of 1 in 1,000 billion even in the fifth generation which is considerably beyond the limits of activity of diluted vaccine virus. Fernet's studies on the morphology of the organism agree in the main with those of Belin, Prowazek, and Volpino. Fernet's method of securing a bacteria-free vaccine virus is believed by Voigt³¹ to have little commercial value, since etherized virus will not retain its potency as long as the ordinary glycerinated product, so that whatever is gained by a shortened period between preparation and distribution is lost by a correspondingly shorter period of potency after marketing. Foster³² secures a marked reduction in the number of bacteria by bubbling ether through a long column of vaccine pulp for a few hours. He does not continue the process until the vaccine is bacteria free, but depends on the glycerin to complete the process during the period of storage which is considerably shortened by this method. This vaccine virus has been in use in the University of California Infirmary for the past year and one hundred per cent of "takes" in previously unvaccinated persons with no history of smallpox testify to its potency. Noguchi³³ has recently succeeded in restoring the virulence of vaccine virus which had been freed from bacteria by etherizing according to the method of Fernet. He inoculates the testicles of rabbits and bulls thereby securing large amounts of protected epithelial surfaces for the propagation of the virus.

A summary of our information regarding the organism of smallpox presents the following points:

1. The organism is present in the lesions of smallpox and vaccinia.
2. The organism is a filterable virus normally occurring as a coccoid form measuring from 0.2 to 0.5 micron in diameter.
3. The organism invades epithelial cells and derives an enveloping substance from the nuclei of these cells.
4. The organism is capable of inoculation in animals though affected somewhat in its general activity by the tissue selected for inoculation or by the portal of entry into the body.

5. It occurs in skin lesions accompanied by bacteria and the removal of the bacteria is difficult of accomplishment without lessening the activity of the organism.

6. The organism has been grown in pure culture *in vivo*, and in culture media containing epithelial tissue. It has possibly been grown in culture media without epithelial tissue, though this last point needs confirmation.

The organism of chickenpox resembles that of smallpox in its invasion of epithelial cells and the formation of an enveloping substance derived from the nucleus. It has produced inclusion bodies in the corneal epithelial cells of rabbits and has been carried from arm to arm for several generations. Owing to the difficulty of animal inoculation it has never been proved that it would pass through a Berkefeld filter.

DIFFERENTIAL DIAGNOSIS OF SMALLPOX.

Much has been written concerning the clinical differences between smallpox and chickenpox, but the fact remains that the morphological similarity of the two organisms is borne out in the diseases produced by them. In every epidemic of smallpox there are so-called "border line" cases which present great difficulty in diagnosis. Smallpox may occur with non-umbilicated vesicles; chickenpox with eruption on the palms and soles, or characterized by definite umbilicated pustules. "One cannot depend absolutely on the gross appearance of the lesion or on its distribution" (Tyzzer). Since the mild cases of smallpox may give rise to severe cases in other individuals, and since our control procedures are radically different for the two diseases, it follows that a laboratory method of diagnosis would be of value to the health officer especially in dispute with physicians unwilling to accept the bedside diagnosis of smallpox.

The following methods of laboratory diagnosis have been suggested at various times:

1. Microscopical examination of the vesicle contents. According to Tyzzer a diagnosis of chickenpox may be made at the bedside by finding the large polynuclear cells which he considers characteristic.

2. Excision of the skin lesion and histological examination. This procedure presupposes a certain degree of familiarity with the not too constant differences in the histology of the invaded epithelial cells. If the diagnosis be made on the basis of inclusion bodies it must not be forgotten that very careful technic is necessary to differentiate these extremely similar bodies.

3. Inoculation of the cornea of a rabbit with the vesicle contents. In this method cover glass preparations may be made directly from the corneal ulcer. The epithelial cells which adhere to the cover glass may be fixed, stained, and examined for inclusion bodies. Paul³⁴ varies this technic by examining the cornea with a hand lens thirty-six hours after scarification in order to detect "air blisters" characteristic of smallpox. Failing to find these "air blisters" he enucleates the eyeball at the end of forty-eight hours and immerses it in a mixture of mercuric chloride and alcohol. The necrotic spots appear as white circular punctures. Neither the "air blisters" nor the necrotic spots are found when chickenpox material is used. Paul admits that the "air blisters" are easy

to overlook and extremely careful technic must be used in enucleating the eyeball in order to avoid smearing the cornea with blood.

4. The inoculation of a well vaccinated person with the contents of the vesicle. Jenner³⁵ in his original series of experiments noted that a sudden efflorescence was produced on the application of smallpox material to the scarified skins of persons who had suffered from smallpox, variola inoculata, or vaccinia. Tieche,³⁶ appreciating the technical difficulties of the usual laboratory diagnosis of smallpox, suggested that these cutaneous allergic phenomena would be present if smallpox vesicle contents were inoculated into the scarified skin of a well vaccinated person, while no result would follow a similar inoculation with chickenpox vesicle contents. Any danger of accidental infection could be overcome by heating the suspected material to 70° C. for five minutes. The fact that Kling had secured typical chickenpox vesicles by arm-to-arm inoculation did not disturb Tieche for, in a later communication,³⁷ he argued that the chickenpox vesicles did not appear for several days after inoculation while the allergic reaction due to smallpox would appear in twenty-four to forty-eight hours. It would seem that Tieche has overlooked one very important factor in the production of cutaneous allergy. Since the chickenpox organism affects epithelial structures, is contained in the vesicle contents, and can be transmitted from arm to arm, it follows that cutaneous allergic phenomena are theoretically possible in response to the varicellation of a person immune to chickenpox. Therefore, unless Tieche secured persons for his test subjects who had neither had chickenpox nor been varicellated, his observations would be open to confusion.

5. Inoculation of vaccinia immune rabbits with the contents of the vesicle. This method suggested by Force and Beckwith³⁸ retains the simplicity of the method of Tieche while guarding against the possible error due to the use of human subjects. Since chickenpox has never been transmitted to the skin of a rabbit, repeated inoculation with chickenpox material will not result in allergic reactions. On the other hand, rabbits sensitized by vaccination with vaccine virus will give a marked intradermal reaction with smallpox vesicle contents in from twenty-four to forty-eight hours. The original vaccination may be either cutaneous or subcutaneous and the cutaneous allergy in some rabbits persists over a year. The reaction has been produced with smallpox vesicle contents nine days after removal from the patient which would allow time for transportation to central laboratories where immune animals might be kept. Intradermal inoculation with 0.1 c.c. of a 1 in 10 dilution of smallpox vesicle contents is followed by the appearance within twenty-four hours of an indurated reddened nodule measuring from 10 to 25 m.m. in diameter. The reaction reaches its maximum development in forty-eight hours. In speed, in simplicity, and in the positive character of the results this method should commend itself to the laboratory worker as a substitute for the more exacting technic of inoculation on the rabbit's cornea.

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LABORATORY METHODS

Methods for the Determination of Glucose in the Blood

METHOD OF SHAFFER.¹

FIVE c.c. of blood are withdrawn from an arm vein. A few grains of powdered potassium oxalate are placed in the pipette to prevent clotting, as suggested by Folin and Denis, the tip of the pipette being inserted in the rubber tubing attached to the needle. The blood is run at once into an Erlenmeyer flask of 100 to 200 c.c. capacity containing 25 c.c. of water. The pipette is rinsed out twice with the same water. If duplicate determinations are desired, 10 c.c. of blood are drawn and the amounts of water and the other agents are doubled.

"The removal of proteins. For the removal of proteins the flask, covered with a small watch glass, and held at the neck with a test tube holder, is heated quickly over a small flame just to boiling. During the heating the flask should be rotated gently; evaporation is to be avoided. A few drops of dilute acetic acid are at once added to the hot liquid to produce a visible coagulum. Five c.c. of colloidal iron solution (iron dialyzed, Merck) is next added and the mixture well shaken, after which about 0.2 gram of powdered sodium sulphate is added and the mixture again shaken for about ten seconds. The liquid is then poured as completely as possible into a 50 c.c. centrifuge tube and centrifuged at moderate speed for one or two minutes. The water-clear liquid is then poured off through a small filter and 21 c.c. equivalent to 3 c.c. of blood are measured into another 50 c.c. glass centrifuge tube. Twenty-one c.c. of mixed Fehling's-Allihn solution is then added and the tube immersed in boiling water, to the height of the liquid inside the tube, for ten minutes.

"The centrifuge tubes are of rather heavy glass, but are fairly resistant to even such sudden changes of temperature. It is, however, important that they should not be in contact with metallic supports when plunged into the hot water. I have found very satisfactory and convenient for this purpose circular racks, made similar to test tube racks, but constructed of 'fibre board' which is very resistant to the hot water. A rack about 15 c.m. in diameter has space for six tubes and of course permits the heating of all at one time."

"On removal from the water bath the tubes are placed in the centrifuge (each pair being balanced by the addition of water if necessary) and centrifuged for two to four minutes, which packs the cuprous oxide in the bottom of the tube. The liquid is then carefully poured off as completely as possible without loss of oxide. This should be done over a white evaporating dish and in a light place which permits one to see when the oxide is disturbed. The tubes are then partly filled with cold freshly boiled water, the pairs balanced

and once more centrifugated for two or three minutes, after which the wash water is poured off as before. The cuprous oxide may now be determined in either of two ways, both of which are satisfactory.

“Colorimetric determination of cuprous oxide. The sediment of cuprous oxide is dissolved in the smallest possible amount of concentrated nitric acid (2 to 10 drops) and then is diluted with ammonia water (1 part strong NH_4OH to 5 parts water) to such a volume that the blue color is roughly about the same as the standard. Cylinders (10 c.c. to 50 c.c.) may be used for this dilution, though of course the reading of the volume should be accurately made. The unknown is then read against the standard copper solution, the latter set at 20 m.m. in a Duboscq colorimeter.

“The standard ammoniacal copper solution contains 5 mgm. of copper in 10 c.c. and is prepared by dissolving 9.82 grams of crystalline copper sulphate in 500 c.c.; 10 c.c. of this solution is diluted to 100 c.c. with 1 to 5 ammonia, which gives a solution that may be easily and accurately read in the colorimeter. I find it best to set the standard at 20 m.m., though from 10 to 30 m.m. it reads almost equally well. The unknown should be between the limits of from half to twice the color of the standard; within these limits the dilution is without much influence. The amount of copper in the unknown is found from the following formula:

$$\frac{\text{St.} \times 5 \times v}{R \times 10} = \text{mgm. Cu. found,}$$

in which St. = reading of standard, R = reading of unknown, and v = volume of unknown.

“This method has been found quick and convenient, though not especially more so than the Bertrand titration as described below. The results agree within 2 or 3 per cent.”

“Determination of copper by Bertrand titration. The washed cuprous oxide contained in the centrifuge tube is dissolved by adding about 0.5 c.c. of strong ferric sulphate sulphuric acid solution,² and is immediately titrated in the centrifuge tube with $\frac{N}{20}$, $\frac{N}{50}$ or $\frac{N}{100}$ potassium permanganate, depending upon the amount of cuprous oxide present. The small amount of liquid in the tube permits a sharp end-point to the titration even with very dilute permanganate. The permanganate should be freshly prepared from a standardized $\frac{N}{10}$ stock solution. One c.c. of $\frac{N}{10}$ permanganate is equivalent to 6.36 mgm. of copper.

“Corrections and calculation. Practically all preparations of Fehling’s solution deposit small amounts of cuprous oxide when heated. Blank determinations of 20 c.c. of Fehling’s solution with 20 c.c. of water heated in a boiling water bath for ten minutes usually yield the equivalent of about 1 mgm. of copper. Such blank determinations must be made on the solution used and the amount subtracted as a correction.

“For the conversion of the result in terms of copper into milligrams of glucose the following table is used. The table has been prepared from the results of a large number of determinations of pure glucose solutions. The amount of copper found, minus the correction, divided by the corresponding factor, equals milligrams of glucose in the blood represented by the amount of filtrate used.

For example, 21 c.c. of blood filtrate, representing 3 c.c. of blood, gave a titration of 5.5 c.c. $\frac{N}{10}$ permanganate."

$5.5 \times 1.27 = 6.99 - 1.2 \text{ (Correction)} = 5.79.$
 $5.79 \div 2.19 = 2.64 \text{ mgm. glucose or } 0.088 \text{ per cent in the blood.}$

TABLE I.
STANDARD COPPER: GLUCOSE VALUES.

20 c.c. of sugar solution plus 20 c.c. Fehling's solution heated 10 minutes in a boiling water bath. Mgm. Cu. — divisor = mgm. glucose.

MGM. COPPER	DIVISOR	MGM. COPPER	DIVISOR	MGM. COPPER	DIVISOR
0.7	1.50	6.0	2.19	20.0	2.06
1.0	1.60	7.0	2.18	25.0	2.04
1.5	1.70	8.0	2.17	30.0	2.03
2.0	1.80	9.0	2.16	35.0	2.01
2.5	1.90	10.0	2.15	40.0	2.00
3.0	2.00	12.0	2.14	50.0	2.00
3.5	2.10	14.0	2.12	60.0	1.99
4.0	2.20	16.0	2.10	80.0	1.98
5.0	2.20	18.0	2.08	100.0	1.97

METHOD OF KOWARSKY AS MODIFIED BY STROUSE.

Strouse, Stein and Wisely have recently reported a modification of the Kowarsky method which requires the use of only 0.5 c.c. of blood for each determination.

SOLUTIONS.

- I. Copper sulphate (pure crystals)..... 8.0
Dextrose (C. P.) 0.1
Water to 200.0
- II. Pure Rochelle salts 40.0
Sodium hydroxide (sticks)..... 30.0
Water to 200.0
- III. Ferric sulphate 10.0
Concentrated sulphuric acid..... 40.0
Water to 200.0
- IV. Potassium permanganate 1.0
Water to 200.0
- V. Liquor ferri oxidati dialysati (Merck).... 5%
- VI. Sodium fluoride 0.2% solution
Also pulverized Rochelle salts.

"Solution IV is used in a 10-fold dilution freshly prepared each day. It must first be standardized by titration against oxalic acid. The equivalent of the permanganate solution in copper is obtained by this titration. Kowarsky recommends the following technic of standardization. Exactly 0.1 gm. of ammonium oxalate is dissolved in 100 c.c. of distilled water. To 10 c.c. of this solution 2 c.c. of concentrated sulphuric acid are added. The diluted permanganate solution is now titrated against this oxalic solution to a permanent red reaction. The number of cubic centimeters of permanganate solution is then made the denominator of a fraction of which the constant 8.95 is the numerator. The resulting figure equals the amount of copper in milligrams represented by 1 c.c. of the permanganate solution.

Apparatus Needed.—"1. Several centrifuge tubes of ordinary laboratory size carefully calibrated and marked at the 0.5, 1.0, 5.0 and 10.0 c.c. points. We

have found it advisable to calibrate our own tubes, as several supposedly accurate tubes showed errors as high as 40 per cent.

"2. An ordinary laboratory centrifuge.

"3. Small Erlenmeyer flasks—capacity 50 c.c.

"4. An Allihn filter and suction pump.

"5. Washed asbestos.

"6. Burette of 10 c.c. capacity divided into $\frac{1}{20}$ c.c. (0.05).

"7. Volumetric flasks, 2, 3 and 5 c.c. pipettes, etc., as found in any laboratory.

The Method.—"The sodium fluoroide solution is placed in one of the calibrated centrifuge tubes up to the 0.5 c.c. mark. In practically all of our work the blood from a finger or ear prick was allowed to drop into the tube up to the 1 c.c. mark, thus giving exactly 0.5 c.c. blood. Water is then added up to the 5 c.c. mark, a pinch of powdered Rochelle salts dropped in, the whole shaken and allowed to stand until the Rochelle salts are completely dissolved. Then the solution of dialysed iron is added, making the total contents of the tube exactly 10 c.c. It is now corked and vigorously shaken until the originally putty-like mixture becomes homogeneous and fluid. Centrifugalize for 3 to 5 minutes.

"The dialyzed iron-blood mixture when removed from the centrifuge should show a water-clear supernatant fluid. Exactly 5 c.c. of this clear fluid is used in the copper reduction, which is performed by mixing exactly 2 c.c. of each of solutions I and II in the small Erlenmeyer flask and then adding the clear filtrate.

"The reduction process must take place on an asbestos mesh over a low constant flame and must continue exactly 3 minutes from the time boiling starts. While this is going on, the asbestos filter is prepared and washed, and the suction pump thereby tested out. After 3 minutes' boiling the Erlenmeyer is rapidly cooled under the cold water faucet without shaking, and the contents immediately poured on the filter. The reduced copper oxide remains in part adherent to the sides of the Erlenmeyer flask and to the surface of the asbestos filter, and both flask and filter must be carefully washed several times with distilled water.

"The funnel is then disconnected from the filtrate flask containing the unreduced copper sulphate and the washings. The filtrate flask is thoroughly cleansed with distilled water and again connected up with the funnel. Three cubic centimeters of the acid ferric sulphate solution are now added to the original Erlenmeyer flask, care being taken that it is well distributed over the whole of the inside of the flask. The contents of the flask are then poured upon the asbestos in the funnel and allowed to stand one minute. By this time all the reduced copper will have been taken into solution and the suction pump is again started. The Erlenmeyer flask is carefully washed 3 or 4 times with 3 to 5 c.c. of distilled water, each washing being poured into the funnel of the suction pump. The contents of the filtrate flask are then titrated against the permanganate solution diluted 1:10, until a definite pink color persists.

"The results are easily figured. The amount of permanganate solution in cubic centimeters is multiplied by the constant for this solution (as previously

determined) and its equivalent in milligrams of dextrose obtained from the following table.

COPPER	SUGAR	COPPER	SUGAR	COPPER	SUGAR
1.95	1.0	3.50	1.8	5.00	2.6
2.15	1.1	3.70	1.9	5.20	2.7
2.35	1.2	3.85	2.0	5.40	2.8
2.55	1.3	4.05	2.1	5.60	2.9
2.75	1.4	4.25	2.2	5.75	3.0
2.90	1.5	4.45	2.3	5.95	3.1
3.10	1.6	4.65	2.4	6.15	3.2
3.30	1.7	4.80	2.5	6.35	3.3

"But it must be remembered that 200 c.c. of the original copper solution contained 100 mg. of dextrose, so that the 2 c.c. used in the test contained 1 mg. of sugar. When this is subtracted from the sugar obtained, we have just the amount of sugar in the blood. Originally 0.5 c.c. of blood was used, but only half the total (5 c.c. filtrate) is used in the test, so that the amount of sugar represents that in 0.25 c.c. of blood. From this it is easy to determine the amount in 100 c.c. of blood, by multiplying by 400.

"It is well here to call attention to the fact that for the titration to be successful the filtrate, after the addition of the dialyzed iron and centrifugalization, must be water clear. Two factors occasionally enter to prevent this. At times not enough Rochelle salts is added and at other times there is insufficient iron. To obviate the latter possibility we have been in the habit of using 5 to 6 c.c. of the iron solution. Students of the original Kowarsky article will note that he advises the use of 'Merck's liquor ferri dialysati oxidati diluted with an equal amount of water.' However, the liquor put out in this country is a 5 per cent solution, and must be used in its whole strength. Dilution according to Kowarsky's technic will spoil the test. At times the iron solution does not seem to maintain a constant strength, and often in the case of a new bottle it will be found necessary to use a little more or a little less than 5 c.c.—always being careful, however, that the total equals 10 c.c."

—R. S. M.

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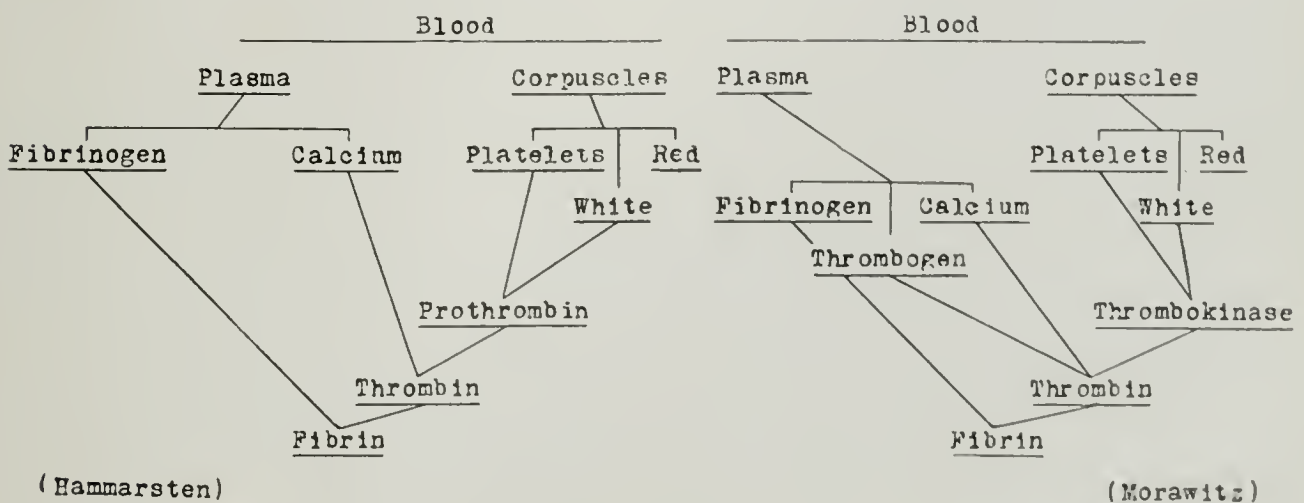
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The Coagulation of the Blood

THE scientific investigation of the process of blood coagulation dates from the work of Hewson in the latter part of the eighteenth century. From his time down to the present many contributions have been made to the study, but the exact stages and factors concerned in the process are still matters of controversy.

Hewson found that the formed elements of the blood take no part in the formation of the clot. Buchanan showed that coagulation is a change of soluble fibrinogen into insoluble fibrin by the action of a second substance. Schmidt concluded that the process is the action of fibrin ferment (thrombin) on fibrinogen, the ferment being activated from prothrombin to thrombin by the action of a zymoplastic substance from disintegrating cells. Wooldridge laid stress on the importance of disintegration products of blood platelets, but he did not regard coagulation as a ferment action. Green, Arthus and Pages discovered the necessity of calcium salts, while Pekelharing and Hammarsten showed that the action of lime salts is on prothrombin and not on fibrinogen.

These studies have led to a theory which is generally accepted at present: Fibrinogen, a soluble proteid of the plasma is converted into insoluble fibrin by the action of thrombin; this is produced after the blood is shed by some precursor from the disintegration of leucocytes or blood platelets. Calcium salts are essential to the process. This is the basic theory at present, but recent workers disagree concerning both the formation of thrombin and the character of its action. Some maintain that prothrombin is changed to thrombin by the action of calcium salts. Among these are Hammersten, Pekelharing, and Rittger who concluded that prothrombin is produced by the disintegration of leucocytes and blood platelets. Howell believes that the prothrombin is present as such, but antithrombin inhibits the action of the calcium salts on it. A more elaborate theory of the formation of thrombin has been worked out by Morawitz, who found that thrombin is produced by the action of thrombokinase (from the disintegration of blood platelets and tissues) on thrombogen (a substance in solution in plasma) in the presence of calcium salts. The difference in the two theories can be made clearer diagrammatically.



The ferment or non-ferment character of thrombin is another point of dispute. Morawitz and his followers regard thrombin as a true ferment because it is not used up in converting fibrinogen to fibrin. Nolf and Rittger, however, state that the action of thrombin is not that of a ferment. According to Nolf, clotting is due to the interaction and precipitation of colloids. Rittger says fibrin is a simple union of fibrinogen and thrombin, and kinases act as particles of foreign matter in hastening coagulation.

The process of blood coagulation is still obscure but the research in this field has resulted in much information that has been given practical application in arresting hemorrhage, both in normal persons and in hemophiliacs. Numerous remedies for hemorrhage have been made use of, all of which have been found beneficial in some cases, and totally inadequate in others. Tannic acid, digitalis, ergot, epinephrin and gelatine have been commonly employed. Gelatine is used locally, but the process of heating it for sterilization deprives it of much of its action. It is more effective given by mouth when it is possible for treatment to extend over several months. There is one case on record where the blood of a hemophiliac coagulated so readily after eighteen months' treatment¹ that no blood test could be made. Röntgeno-therapy has been successful at times, but it has frequently been misused. Intravenous injection of sugar solution² is another method which has brought results, its benefit depending on a modification of osmosis. It has the advantage over salt solution because it is nutritive, and over blood serum because there is no danger of anaphylaxis.

It was early observed that injured tissue cells liberate a substance which accelerates coagulation, but whether this is of the nature of thrombokinase or of a thromboplastin which neutralizes antithrombin, is still undetermined. It is stated that the combined action of tissue and blood coagulins produces a greater effect on coagulation than the sum of their separate actions. Hemophiliacs often present a lack of thrombokinase in the blood but it does not always follow that there is a corresponding lack in the tissues. Hence tissue juices from the patient himself are available. These are used in connection with actual cautery where the benefit is ascribed to the necrosis of tissues, and also in the act of crushing and manipulating tissues around bleeding points.³ Tissue extracts, especially of goiter, lymph glands, liver and others rich in coagulin, are employed locally. Experiments on animals⁴ prove that the intravenous use of these extracts shortens the coagulation time materially, but this method applied to humans has proved disappointing so far. The danger in the intravenous use of these extracts was pointed out by Wooldridge who found that there is a distinct negative phase following injection, its extent and duration depending on the dosage and the condition of the animal. The negative and positive phases are analogous to those following the injection of toxins except that in the case of tissue extracts the phases are brought about within a few seconds. The intravenous injection of peptone solution affords another instance of two opposite phases. Immediately after injection, coagulation is hastened. If death does not result from intravascular clotting there follows a phase in which coagulation is retarded. The increase is thought to be due to the production of a great quantity of fibrin ferment and the decrease may possibly be due to the secretion

by the liver of an excess of anticoagulin. Peptone solution has been tried as a hemostatic, but its use, at least in one instance, was followed by intolerance and death.⁵

In general, the means which has brought the best results is that of supplying blood or one of its constituents. Dried fibrin, human or sheep, has stopped hemorrhage, when applied directly. Calcium is indicated when the calcium content is low as, for instance, in some forms of jaundice. This is supplied most commonly in the form of calcium lactate which is effective given by mouth when the treatment lasts several days.⁶ A prompt though temporary effect is obtained by intravenous injections. The great success that has attended the employment of blood is perhaps due to the fact that besides providing new factors of coagulation it furnishes also nutrition and new immune bodies. Blood is used in large quantities in the fresh form, defibrinated and as serum. Defibrinated blood has been used both subcutaneously and intravenously, the latter method being the most active. The sera commonly employed are horse serum,^{7, 8} even in the form of diphtheria antitoxin⁹ when the normal serum is not available, and human serum.^{10, 11, 12} The disadvantage of animal serum is the danger of anaphylaxis and of contamination and loss of strength if it has been preserved for any length of time. Fresh blood is efficacious if applied directly to the bleeding surface.¹³ More frequently it is transfused from another individual to the patient.¹⁴ This treatment has been used to a great extent recently for hemorrhage in the new born. The blood is injected into the sagittal sinus through the anterior fontanel. In indirect transfusion, the blood is received into an oiled vessel or is mixed with hirudin,¹⁵ and it is then injected into the patient. These procedures retard coagulation. The oiled vessel presents a surface similar to that of the vessel lining and the hirudin acts as an antithrombin. Such a minute quantity is needed that it has no derogatory effects. In direct transfusion blood is passed directly from the vein of the donor to that of the recipient. Formerly transfusion of normal blood of a close relative was advocated, but it is becoming the custom to use entirely foreign blood as this has often been beneficial when the former proved of no avail.¹⁶ It seems that the danger from dried transfusions there were only fifteen instances of macroscopic hemolysis, hemolysis has been exaggerated. One set of statistics¹⁷ shows that in eight hundred and eleven of these cases there was recovery. It is considered advisable to make the test except in instances when the loss of time occasioned would prove dangerous.

These comprise the usual methods for the arrest of hemorrhage. Since the problem of coagulation is still unsolved, it is to be expected that seemingly contrary results will be obtained and will not be wholly accounted for. However, much of the confusion may be attributed to lack of discretion in the choice of treatment. More effort should be made to determine just what factor of coagulation is missing, or if all are present whether there is an excess of antithrombin. For instance, no amount of calcium will help a case where the difficulty lies in lack of prothrombin or fibrinogen, and tissue extracts are not indicated unless the bleeding is due to lack of thrombokinase or excess of antithrombin. One of the reasons why the use of blood has met with such extensive success is that the missing element whatever it is, is supplied from the normal blood.

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ETHYL STOLDT,
(per V. C. V.)

*Harper Hospital,
Detroit.*

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EDITORIALS

Experimental Arthritis in Rabbits

AN extremely interesting study on experimental arthritis in rabbits, published by H. K. Faber in the *Journal of Experimental Medicine*,¹ introduces a novel, and at the same time theoretically sound, point of view into the investigation of rheumatic disease. Past inquiries into the etiology of rheumatism have largely confined themselves to attempts to isolate bacteria from joints or to produce experimental infection in animals by injections of pure cultures systemically, or into the joints themselves, and it is, of course, familiar to physicians that many microorganisms have been held responsible for this protean disease. Loeffler² produced arthritis with streptococci as early as 1884. The work of Poynton and Paine³ with the Gram-negative coccus they held responsible for rheumatism has been too recently under discussion to need comment. Rose-now's work on isolation of streptococci has called particular attention to the problem of bacterial origin of the disease, during the past two years. Achalmé⁴⁻⁸ believed the etiological factor to be a bacillus belonging to the aerogenes capsulatus group. Many bacteriologists have hesitated to assume that acute articular rheumatism was a disease of uniform bacteriological etiology largely for the reason that few experimenters have failed at one time or another to observe joint lesions in systemically infected animals when various different microorganisms were the infecting agents. It has seemed, however, that the relation

of the streptococci to the disease might be a more intimate one than that of any of the other bacteria.

The line of research followed out by Faber was suggested, he states, by the work of Herry⁹ in 1913, who injected into the joints extracts of streptococci, isolated from the blood in cases of rheumatic fever, and fifteen days later followed this with an injection of the living bacteria. Faber calls attention to the interesting fact that in many of the cases of experimental arthritis produced in animals there has been a definite incubation period before the joint lesion occurred, and that Cole,¹⁰ Shaw,¹¹ and later Schloss and Foster,¹² found that arthritis in animals was obtained only after repeated injections. It seemed obvious from this that sensitization of the joints to the microorganisms might be a factor in the selective localization of the infection. In his first experiments Faber used streptococcus viridans strains. The animals used were rabbits ranging from 1,200 to 1,800 grams. When the bacteria were injected intravenously it was found that no arthritis developed after one and two injections, but that at least two preliminary sensitizing doses were needed before any of the rabbits developed arthritis.

In another series of experiments he attempted to sensitize the joints locally. Suspensions of the organisms were injected into the left knee, using care not to infect the periarticular tissues. The result was usually an arthritis which after two to four weeks got well, the joint contents becoming normal. In most cases he used dead bacteria for such sensitization. At varying periods after the joints had become normal, intravenous injections of the same organisms were given. In almost all of fifteen rabbits gross signs of arthritis developed. This seemed to show that sensitization of a joint was possible and that arthritis might develop as a result of repeated inflammation of a joint once sensitized when the same bacterial cause was applied. However, it was still an open question whether or not such sensitization was specific. Crossing with streptococcus and pneumococcus resulted in failure of reaction. However, when closely related strains of streptococci were used for sensitization and subsequent injection, slight reactions (doubtful) were obtained.

The work is extremely interesting in showing that actual sensitization of a joint may bring about a specific reaction that predisposes to subsequent joint inflammations when the homologous organism reinfects the body. It would also suggest that a number of different bacteria can cause arthritis, and that, as Faber correctly suggests, one organism is not always at fault. That the streptococcus, and chiefly that of the viridans group, should be the one most commonly causing the disease in human beings is rather natural owing to the relations of virulence and resistance, the method of invasion of this organism, and its common occurrence in and upon the human body. However, Faber's work opens the possibility that the occurrence of rheumatism, or rheumatic joint infections, depends not only upon the presence of a microorganism capable of invading the joints, but of a previous specific hypersusceptibility of the joint tissues for this organism caused by a preceding inflammation.

Incidentally the work of Herry and of Faber opens up a line of reasoning more broad than its strict application to the explanation of rheumatism. If his views regarding rheumatism should turn out to be correct, there is no reason

why similar sensitization of other tissues of the body may not explain in general the selection of various parts of the body for specific injury when other parts equally exposed to invasion by the microorganisms are spared.

—H. Z.

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Origin and Spread of the Cardiac Impulse in the Auricle

WITH the discovery that it is by the so-called bundle of His and Kent, or the auriculo-ventricular bundle, that the cardiac impulse passes from the auricle to the ventricle, interest soon became aroused as to the exact place in the auricle at which the impulse originates. It had for long been known that, in the cold-blooded heart, the impulse definitely starts at the sinus venosus and spreads over the auricles to the ventricles, and it had also been observed, in the mammalian heart, that the bases of the large veins, where these join the auricles, are likely to continue beating for a considerable time after the rest of the heart has ceased to beat.

The first definite progress in the localization of the site of origin of the beat in the mammalian heart was furnished by the discovery of Keith and Flack of a peculiar type of tissue located at the junction of the superior vena cava and the right auricle. In this position they found a small artery which is surrounded by fibrous tissue containing numerous peculiar muscle fibers, some nerve cells and some nerve fibers. The muscle fibers were found to have a structure not unlike those of the auriculo-ventricular node. These anatomists suggested that it might be in this "sinus node" that the cardiac impulse originated.

To put the hypothesis to the test various methods have been employed. These include: (1) injury of the node in various ways; (2) determination of the comparative rhythmicity of strips of the auricular walls, which contained or did not contain nodal tissue; (3) determination, by the use of galvanometric curves, of the relation of the node to the seat of origin of the cardiac impulse. The conclusions which have been drawn from these investigations have not been entirely in harmony. Lewis, Zahn, Meek and Eyster, etc., have held that the node is the site of origination of the beat, whereas Erlanger maintains that the structure has no initiative not shared by other auricular tissue in the region surrounding it.

Closely linked to the question of its site of origin is that of the course of the excitation wave in the auricles. Does it travel by some specially provided path running between the sino-auricular and auriculo-ventricular nodes, or does it spread uniformly over the whole auricle. If the former should be the mode

of propagation, the impulse will reach the auriculo-ventricular node before the auricles go into contraction. The importance of knowing exactly whether this is the case is evident in connection with the whole question of cardiac arrhythmias.

We shall not attempt to review all of the literature pertaining to this question which has recently appeared, but will confine ourselves to the papers of Eyster and Meek^{2, 3} and of Lewis and his pupils.^{4, 5} But to follow the work of these investigators, it is essential that we understand clearly the principles of the methods which they have employed. These principles are very clearly and simply set forth in the recent Harvey lecture by Lewis. Briefly summarized the main points are these: When a galvanometer is connected by means of unpolarizable electrodes with two parts of a denervated muscle, a current will be found to become established when a wave of contraction passes over the muscle from one end to the other. The portion of muscle that contracts first becomes electrically negative to the rest of the muscle. As the wave of contraction passes along the muscle, the negativity, as it is called, gets less at the end at which the wave started, until, when the wave is in the center of the strip, neither end of the muscle shows any difference in potential, so that the galvanometer will come to rest. As the wave reaches the farther end of the muscle, this in turn becomes negative, and the needle will swing in the opposite direction. It is evident, then, that the initiation of a muscular contraction wave occurs at the electrode which is negative first. Another important observation is that the movement of the galvanometer in the above experiment is most marked when the two electrodes are at the extreme ends of the muscle; that is to say, the amplitude of the negative wave is greatest when the time interval between the receipt of the excitation at the two contacts is greatest.

The application of these facts to the study of the initiation of the beat in the auricle requires that we should consider one other proposition, namely, this: If a pair of contacts be arranged in the center of a circular sheet of muscle, and the edge of this sheet be stimulated at different points, the amplitude of deflection on the galvanometer will be greatest when the contacts are radial to the points of stimulation, for under these conditions, it is evident that the greatest possible interval will exist between the arrival of the wave at the two contacts.

Before proceeding to consider the manner in which these principles may be applied in the location of the pacemaker of the heart, it is important to call attention to the methods which have been employed to ascertain exactly the positions at which the electrodes were placed on the auricle. In Eyster and Meek's work the point of application of the electrodes was marked by ligatures, and after the experiment was over, the heart was examined microscopically so as to locate exactly the position of the ligatures. Lewis was even more careful. Scale drawings were made of the auricle during the experiment, certain landmarks being employed so as to locate the exact position of the electrodes. After the experiment, the auricular portion of the heart was hardened, dehydrated and embedded *en masse* in paraffin. The impregnated auricle was then compared with the original scale drawing and a new drawing was made, upon which the chief muscular bands of the auricle were sketched in their relationship to points of application of the electrodes.

Regarding the point of origin of the beat the following facts indicate that

it is in the sino-auricular node. First, when the contour of the wave is compared in electrocardiograms taken with the two electrodes applied at different parts of the auricle, it is always found that the greatest amplitude of movement is obtained when the line joining the electrodes converges to the sino-auricular node. From experiments with the circular sheet of muscle above alluded to, it is evident that the stimulus to produce this condition must arise in the neighborhood of the node.

Second, if one electrode be placed on the sino-auricular node and another electrode moved about from place to place on the auricle, the electrode on the node is always negative to the other. This is not the case if the electrodes are on any other parts of the auricle.

Third, by comparing normal electrocardiograms from lead 2 (i. e., when the right fore limb and the left hind limb are connected with the galvanometer) with electrocardiograms taken when beats are started by applying artificial stimulation to the various parts of the auricle, it is found that the normal curve is obtained only when the stimulated part is in the neighborhood of the sino-auricular node. This is particularly noticeable in the case of the P-wave, which is the characteristic auricular wave, and is obtained, after artificial stimulation of the auricle, only when this stimulation is at the sino-auricular node. If the stimulation be at the appendix or at the superior vena cava, the P-wave is distorted although the other waves appear normal.

Fourth, by taking simultaneous electrocardiograms from a direct lead on the auricle and a standard limb lead (Lead 2, for example) and determining, by exact methods of mensuration, the time of onset of the excitation wave, relative to P in the standard, in various regions of the auricle, it is found that the first appearance of the excitation wave occurs earlier when one electrode is over the upper end of the sino-auricular node, and that, in other regions of the auricle, it always appears at a later interval.

Fifth, careful examination of electrocardiograms from different parts of the auricle shows that the main wave of negativity is sometimes preceded by slight positive waves, that is, waves that dip below the base line. It will further be noticed that the waves showing no initial deflection are always obtained when one electrode is on the sino-auricular node. Lewis and his pupils, who have investigated this point, have come to the conclusion that the deflection waves preceding the main wave of negativity are really due to extrinsic influences acting on the electrodes. In other words, the electrodes pick up electric discharges from distant areas of muscle, while these are in a condition of contraction. Thus, if electrocardiograms be taken from two leads on the auricular appendix, a very distinct initial deflection will precede the main wave of negativity. If now the base of the appendix be crushed so that no wave of contraction can reach the appendix, the wave of negativity will disappear (that is to say, the intrinsic deflection), but the initial or extrinsic deflection wave will remain, being conducted to the electrode from a distant part of the auricle. "All curves from leads at a distance from the sino-auricular node or outlying leads, as we may call them, therefore give curves of a more or less composite form; consisting of a main deflection, which corresponds to the arrival of the excitation process, and diminutive initial deflections which are due to the passage of other portions of the auricular muscle into the excitatory state." From these considerations it follows that the interval between the intrinsic and ex-

trinsic deflections should be longest in leads that are farthest from the node, and that they should approach as one of the contacts approximates the node, until over this structure, the extrinsic deflection is no longer recorded. Such has been found to be the case. The importance of these observations rests on the fact that previous observers have not sufficiently regarded the influence of extrinsic deflections in interpreting the curves.

Taking all these facts together, then, there remains not the shadow of a doubt that the beat of the heart originates in the upper club-shaped extremity of the sino-auricular node. So far as we are aware, the only authority who has disputed this conclusion is Erlanger, who has pointed out that a very feeble, undetectable negativity might originate somewhere near the node, but only become sufficiently pronounced to show itself when it affected the more reactive tissue of which the node is composed. Eyster and Meek have tried to remove this objection by showing that similar results to those recorded from the outside of the auricle are obtained when the electrodes are applied, not to the exterior wall of the auricle, but to its endocardiac surface.

It is important to note that Eyster and Meek³ have conclusively demonstrated the unreliability of using observations from the dying mammalian heart for the purpose of determining the site of origin of beat. It is of course well known that the power of contraction remains in the venous and auricular regions for a considerable time after the ventricles have ceased to beat. This part—the *ultimum moriens*—in most hearts is situated somewhat lower than the sino-auricular node. Though this is the last part of the heart to cease contracting, that does not mean that it is the part of the heart in which the beat ordinarily originates. It simply means that it is the part of the auricle in which the power of contraction remains for the longest time after death.

The course of the excitation wave in the auricle and the rate of its conduction.—This can be determined by two methods, which we may call the direct and the indirect. In the direct method a series of pairs of contacts is placed on the auricle, each pair being in a radial direction to the sino-auricular node. The time at which the excitatory process arrives at that contact of each pair which is proximal to the sino-auricular node is then accurately determined from the galvanometric record. The exact distance between the contacts and the sino-auricular node is now measured and from the data the average transmission time is estimated.

In the indirect method the onset of the negative wave from different leads is compared against a standard. Considerable difficulty has been experienced in finding a suitable standard for this purpose. Eyster and Meek have used the mechanical systole of the auricle, that is to say, they compare the onset of negativity in any region with mechanical systole of the right auricle. The chief objection to this method is the great difficulty of knowing, from the mechanical record, the exact moment at which this act starts. Lewis and his pupils have also used an indirect method (Sanderson's) which, however, is too complicated for description here. It is admitted by Lewis, however, that this indirect method is applicable only when the contacts are strictly in line with the sino-auricular node, that is to say, on the taenia terminalis.

The results of the above investigators are not in entire harmony with regard, either to the relative rate of propagation of the wave to different portions of the auricle, or to the actual rate at which the wave travels. Lewis maintains

that the transmission rates are uniform from the node to all parts of the auricle, with the exception of the superior vena cava, in which the rate is considerably lower. One thousand millimeters per second represents very fairly the average rate at which the wave travels. Eyster and Meek, on the other hand, state that the wave is propagated throughout the sinus node and spreads to the contiguous venous region and to the auriculo-ventricular node with considerable rapidity—reaching the mouth of the superior vena cava in 0.01 second or less—whereas its passage to the auricle takes longer, namely, 0.02 second. There is, therefore, a delay in the passage of the wave to the auricle, which indicates that the excitation must spread to the auriculo-ventricular node before involving the right atrium. They conclude: "This leads to the inevitable conclusion that the cardiac impulse spreads to the ventricle and to the right auricle by different paths, and does not pass to the ventricle through the auricle, as ordinarily stated."

Lewis and his collaborators place great emphasis on the fact that the determining factor in the spread of the excitation wave is the disposition of the auricular muscle fiber. In their heart specimens, as above described, they could show clearly that the muscle fibers all radiate in a curious fan-shaped manner, from a point which lies immediately below the sino-auricular node, to all parts of the superficies of the right heart. They have named this the *concentration point*. At the end of the vena cava the muscle fibers are arranged more or less circularly, which may explain the delay in transmission which these authors found at this place. The results of their investigations may therefore be summed up by stating that the excitation wave in the auricle spreads in the same manner as a fluid poured upon a flat surface would spread. Its edge advances in an ever widening circle until the whole surface is covered, and the rate of spread is approximately 1000 millimeters per second.

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—J. J. R. M.

Nervous Influences and Edema

THE conception that the nervous system plays a major role in such conditions as inflammation and edema, is a widely held one, chiefly perhaps because there has been, until very recent times, no other explanation which could be offered. And yet there seems to be modern evidence that the nervous system plays only a minor role in either process; that it plays no essential part in their production, but merely increases or decreases the rapidity of their courses. In the absence of nervous impulses, either process will follow its normal course although that may be lengthened.¹

In a recent article Heitz and DeJong² discuss the relation between nervous

influences and edema. They commence by saying that Vulpian showed that section of nerves is accompanied by vaso dilatation which facilitates serous transudation and infiltration of the tissues. They mention the experiments of Claude Bernard, Ranvier and others which perhaps form the basis of the neurogenic conception of edema. They call attention to clinical experiences, in the neuritides, infections, myelitides, encephalitides, and other types of cerebral lesions in which edema occurs, which seem to substantiate the neural conception. They have collected many facts which as they say lead to the conclusion that edema is only a manifestation of the accumulation in the tissues of retained chlorides. They say that the facts lead to the conclusion that edema is a manifestation of the accumulation in the tissues of chlorides which are not eliminated by the kidneys and that vaso-dilatation has only the effect of influencing their distribution in the tissues. But this is not the interesting fact that merits most attention, for whether chloride retention is the cause of edema or not, is a matter of much discussion with, we think, the best evidence in the negative.³ The essential fact that is brought out by Heitz and DeJong is that the nervous system is not an important mechanism in edema. A nervous system lesion, they say, is powerless to cause edema. And when edema arises in the course of a disease of the nervous system, it calls attention to the presence of a coincident renal or cardiac abnormality which up to the time of observation had remained unnoticed.

These conclusions rest upon the observations of cases, which are quoted, of cerebral lesions accompanied by hemi-edemas, and the authors state, it is just such cases which have formed the main basis of the neural doctrine of edema.

Just why the edema should affect the paralyzed limbs is not stated. It might be suggested that the cause of this is the decreased circulatory activity in the affected tissues, which prevents normal oxidative processes and which also shows the rate of removal of waste materials.

¹Woolley: Jour. Amer. Med. Assn., 1914 (63) 2279; also Centr. f. allg. Path., 1915 (26) 218.

²Heitz and DeJong: Arch. d. Mal. du Coeur, 1915 (8) 148.

³For the arguments see Fischer: Oedema and Nephritis, New York, 1915 p. 525, 529, etc.

—P. G. W.

Ethylhydrocuprein and Pneumococci

HENRY F. MOORE, working at the Rockefeller Institute for Medical Research,¹ has again studied the bactericidal effect of ethylhydrocuprein on pneumococci. This substance was first introduced by Morgenroth² who found that it had a more or less specific antagonistic action for pneumococci. The same results were achieved by A. E. Wright³ and others, and Moore's observations further confirm them. Moore used rabbits as his test animals. He gave these animals subcutaneous injections of ethylhydrocuprein, dissolved in distilled water or sterile olive oil, finding that a dose of 0.1 gram per kilo of body weight was well tolerated. It appears from his protocols that doses exceeding this amount usually were toxic. An animal receiving 0.2 grams per kilo showed severe toxic symptoms, as did one receiving 0.15. Animals receiving 0.125 in several cases showed "a tendency to lie quiet for some hours after the injection." When the base of the drug, dissolved in oil, was given intramuscularly it was more toxic than

when given subcutaneously, and the bactericidal effect on the pneumococci noted in the serum was neither so prolonged nor intense as when the drug was subcutaneously administered. The tolerance for the drug was greatest when the hydrochloride, dissolved in distilled water, was put into the stomach, but there were practically no bactericidal effects noted in the serum in these cases.

The serum of animals treated with ethylhydrocuprein subcutaneously, exerted a distinct bactericidal action on pneumococci. The effects were greatest, as stated before, when the base (optochin base) was given subcutaneously in oil. The intravenous route was found to be very toxic, and even in such cases the effects were not prolonged. Bactericidal action of the serum was at its highest point about an hour after the drug had been given. When given to man by mouth in doses of 0.5 grams (of the ethylhydrocuprein hydrochloride), no toxic symptoms occurred. The same dose given in sterile distilled water subcutaneously had no serious effects, though slight pain in the legs, a slight rise of temperature, and a little hyperemia at the site of injection occurred in one case; in the other case there was a slight headache, with similar infiltration around the area of injection, which disappeared within a few days. The serum of individuals to whom such a single dose of the drug had been given had a definite inhibitory effect on pneumococcus growth which lasted for a number of hours, the bactericidal action being much more feeble than in the case of the rabbits.

Moore, very wisely, draws no conclusions concerning the therapeutic possibilities of the drug in pneumococcus infections, but states the facts as he found them. It would seem that his experiments definitely confirm the claim that this substance lends a decided bactericidal power for pneumococci to the serum of animals and of man receiving it, but the small margin between effective amount and toxic dose and the short duration of the bactericidal effects would seem to indicate that in the present form no great therapeutic developments can be hoped for.

¹Jour. of Exper. Med., Nov. 1, 1915, xxii, p. 551.

²Berl. klin. Wchnschr., 1911, xlviii, 1560, 1979.

³Lancet, 1912, ii, p. 1633, 1701.

—H. Z.

Intestinal Stasis

INTESTINAL stasis is fundamentally a mechanical problem, no matter what its sequelæ may be, and it is the mechanical problem that one must solve if one is to understand the underlying causes. A healthy, normal bowel will not permit of stasis, but will carry on its motor functions with ease and regularity. When one says normal bowel, one postulates normal conditions of living.

But, it happens, stasis of greater or less degree occurs in individuals who are living an apparently normal existence. No longer is the regularity of the gut maintained; no longer is its function carried on automatically. To account for these abnormal states it is said that the musculature of the bowel has lost its tonicity, which means that it is not able to contract as powerfully, or that it does not contract rhythmically as it should. It is said that kinks occur (congenital or acquired) and that these, usually due to bands or adhesions, pre-

vent the even progress of the intestinal contents to their normal destination. As a result of such a condition, the intestine becomes dilated, the muscle fatigues so that it no longer is able to do its duty, and then absorption of bacteria or toxic substances takes place with subsequent damage to health. When such anatomic distortions are severe, they interfere with the functions of the bowel, no doubt, but when they are less severe, and this is the rule, they do not interfere to any great extent with the motor functions, and since in very many cases there is no external or visible reason for the stasis, something intrinsic must have gone wrong. To say that the muscle is atonic means nothing.

Recently Keith¹ has given, in the Cavendish Lecture, a possible, and plausible explanation of stasis which reaches deeper into the subject than any other one. It will be remembered that Keith has studied intensively the "nodal" and "bundle" tissues of the heart, and that upon his researches with those of other writers our working knowledge of the controlling muscular mechanism of the heart is based. Keith has interested himself more recently in that part of the gastro-intestinal tract which he believes is analogous to the cardiac controlling system. This is the myenteric (Auerbach's) plexus. This plexus Keith has studied in a large series of human specimens as well as others taken from various other mammals, and his studies have convinced him that it is not a simple structure composed merely of nerve fibres and nerve cells. These it contains, but besides these, are branching intermediate cells which appear to become continuous with processes of certain groups of muscle cells on the one hand, and with the branched processes of ganglionic cells contained in the nerve fibres of the plexus, on the other.

Upon the basis of these observations, Keith conceived the idea that the myenteric plexus is analogous to the nodal and conducting system of the heart;—that as in the heart the efferent nerves which influence the contractive tone and rhythm of the bowel end, not in the muscle, but in the myenteric plexus, which in its turn effects the contractions of the musculature of the gut.

In the course of his researches Keith found that there are certain points in the course of the gastro-intestinal tract where the conducting system is collected into rather larger masses than is the general rule. The points correspond to the nodes of the heart. Such nodes were found at the gastro-esophageal junction, where gastric contractions are initiated; in the ileo-caecal region; and in the rectum. There appears also to be an initiatory point in the duodenum proximal to the entrance of the common bile duct. From each of these areas the fibres of the myenteric plexus seem to extend distally. Between each of these areas the rhythm gradually decreases.

In applying these observations to intestinal stasis Keith says that "in passing along the alimentary tract, food is propelled through a series of zones or segments, each furnished with its own pacemaker and its own rhythmic contractions. In the heart we find two such zones—an auricular and a ventricular; in the normal heart the sino-auricular node is the master pacemaker. But a block or imperfection in conduction may occur between the two zones of the heart, with the result that 'back pressure'—a venous stasis is produced." He supposes that irregularities may occur in the same way in the nodal and conducting mechanism of the alimentary canal, and when they do, he finds that they occur where they should be expected—at points where one rhythmical zone passes into the succeeding one. Blocks are found at the gastro-esophageal

junction, at the second part of the duodenum, at the duodeno-jejunal junction, at the ileo-colic. Not only must these nodes be healthy but they and their rhythmic zones must be closely co-ordinated. Disturbance in any one segment upsets the rhythm in all segments. Distention of the duodenum inhibits the action of the ileum; duodenal disturbances upset the rhythm of the stomach; disease of the myenteric tissue of the caecum will produce abnormalities in the movements of the lower ileum.

Finally Keith says that radiograms have failed to produce evidence of obstruction situated at bands or kinks; there is no hypertrophy of the muscular coats above the bands or kinks; and where acute flexures are produced experimentally, stasis does not result. On the other hand, there is good evidence which points to a gross derangement in the action of the musculature of the whole colon in stasis of the great bowel.

In this material of Keith we have for the first time a truly physiologico-anatomic reason for stasis, but a reason which must be studied and confirmed. If there is such a conducting and co-ordinating neuro-muscular (myenteric) mechanism, how is it deranged? In the heart we find that a block may arise because of a lesion of one of the nodes, or of the branches of the bundle itself, or of the myocardium alone. These lesions may be the result of inflammations or degenerations. Edemas of the tissues or cellular infiltrations of the tissues about the bundle may act by compressing it; drugs carried to the bundle by the blood may modify it; and influences acting by way of the vago-sympathetic system may increase or decrease its powers of conduction. It is perfectly possible that in acute intestinal infections the myenteric plexus is temporarily damaged, and that in chronic inflammatory processes it is permanently affected so that the normal rhythm is interfered with so that perhaps, as in the heart, only every other impulse to contraction is effective. And so one might proceed in drawing the analogy. Keith's is a very suggestive work.

¹Keith: *Lancet*, 1915 (2) 371. See also *Brit. J. Surg.*, 1915 (2) 576.

—P. G. W.

Death by Hanging

IN hanging death is generally assumed to be due to asphyxia, although in a small per cent of the cases of official execution by this method the neck may be broken, and in some rare instances still greater injury to the tissues, both the soft and the bony parts, may be done. Indeed, there are recorded cases in which the head has been completely severed by hanging, the interval between the drop and death is quite variable, depending upon the completeness with which the windpipe is closed and the distance of the drop. In old people there may be some ossification of the trachea, and this quite naturally would interfere with the action of the ligature, and death would be slow in proportion to the extent of the hardening of the tissue. Displacement of the second cervical vertebra occurs rarely, but has been observed when corpulent persons are executed, or when a long drop is given. The location of the noose has something to do with the speediness with which death results. When the cord is placed below the cricoid cartilage asphyxiation follows more speedily than when pressure is made upon the larynx, because with the ligature in the former location

the air is more completely shut off. With the ligature properly placed and with a drop of six or more feet, death by hanging appears to be practically instantaneous, and free from physical suffering. It is true that in practically all cases of legal execution by hanging there are violent movements or convulsions of the trunk and extremities after the body drops, but these are probably unconscious and unaccompanied by pain. When compression of the windpipe is complete and the weight of the entire body is placed on the cord, death certainly occurs within two or three minutes, and after this time resuscitation is impossible. In many cases of attempted suicide by hanging, resuscitation may be brought about after a much longer period, but in these instances the loop has not been adjusted with skill, and in the great majority of them the body is partially supported in some way.

Descriptions of the external appearances after death by hanging have for the most part been taken from observations made at legal executions, and in these cases the face is livid, frequently distorted, sometimes swollen. The eyes are glaring, red, and sometimes markedly protruding from their cavities. The tongue is swollen and either protruding or clenched between the teeth. A frothy mucus, stained with blood, drops from the mouth, the line of the cord around the neck is marked, showing in many instances laceration of tissue, and in others numerous ecchymoses. The hands are clenched, and the lower limbs usually flexed. While these appearances are quite marked and constant in cases of legal execution by hanging, they are usually much less distinct when suicide has been attempted by this method. In suicidal cases the face is frequently pale. There may be no protrusion of the eyes. The line of the cord varies greatly, and in proportion to the weight placed upon it. If the body be partially supported the cord may leave but slight evidence of its location. The nature and width of the cord markedly influence the extent to which violence to the tissue is done. If a suicide hangs himself with a towel, there may be in fact no noticeable mark about the neck. While the hands are usually clenched in legal execution by hanging, this is by no means universally the case in suicides.

On postmortem examination after death by hanging there is found in all the abdominal and thoracic organs and in the brain, as well, marked venous congestion, and as is true in other forms of death from asphyxiation, the right side of the heart is more commonly distended and more completely filled than the left side, and the blood in the heart is frequently fluid, although this is not constantly the case. Venous congestion is often marked, and cerebral hemorrhages are by no means uncommon, but are more frequently found after violent hanging than in suicides.

The expert may be asked to determine whether or not death was induced by hanging. In answering this question he must rely very largely upon evidence furnished by the pressure of the cord. It has been shown experimentally by Devergie that if a corpse immediately after death be suspended by a cord around the neck, redness and even ecchymoses along the line may be induced, and Vrolik showed that when a body was thus suspended one hour after death a distinct livid line, corresponding with the course of the cord, might remain. It follows from this that so far as the mark of the cord is concerned there may be cases in which the expert can only say that the cord was applied during life or immediately after death. Of course, where great injury has been done, and

blood has flowed from penetration of the tissue there can be no hesitancy in deciding that death was caused by hanging, but the fact remains that there may be instances where the evidence furnished by the line of coloration caused by the cord leaves one in doubt. It has been claimed by some that the mark of the cord serves not only in deciding the point whether or not death was due to hanging, but the more difficult question as to whether the case was one of suicide or one of murder. It has been claimed that when the mark of the cord is circular and runs along the lower part of the neck the case could be only one of murder. However, there have been several cases of undoubted suicide in which the mark made by the cord was situated in exactly the place and had the form which it had been claimed could not occur in suicide. In one or two celebrated cases, notably that of the death of the Prince de Condé, in 1830, it was argued that the position of the dead body indicated homicide, and in fact was such that the case could not be one of suicide. This claim was evidently founded upon error. It was argued that death from hanging could not occur when any part of the body rested upon a support. The body of the Prince was found to be partly supported, and it was claimed that he did not die from hanging, but that he was murdered, and his murderer had partially suspended the body in order to make it appear that he had committed suicide. A person may hang himself and still stand on the floor, sit in a chair, or partially lie in bed. It is only necessary to put the proper noose around his neck and to throw the weight of a portion of his body on the cord, in order to commit suicide by hanging. A man may stand on the floor, put a noose about his neck, attach it to some object above him, and then take his feet from the floor, flexing his legs, and hold himself in this position until he becomes unconscious and unable to stand, and when his feet drop to the floor the weight of his unconscious body is sufficient to complete the death which he has consciously undertaken. Then, if the body remains in this position until rigor mortis comes on, it may appear that the man stands with a loose noose about his neck, and without any tension on the cord. The following interesting case is reported by Taylor: "In 1832 a man was found hanging in his room with his knees bent forwards and his feet resting upon the floor. He had evidently been dead for some time, since cadaveric rigidity had already commenced. The manner in which this person (a working mechanic) had committed suicide was as follows: He had made a slip knot with one end of his apron, and having placed his neck in this he threw the other end of the apron over the top of the door, and shutting the door behind him, he succeeded in wedging it firmly. At the same moment he had probably raised himself on tip-toe and then allowed himself to fall. In this position he died. The weight of his body had already sufficed to drag down a part of his apron, for it seemed as if it had been much stretched. The deceased was in the position in which the body of the Prince de Condé was found, and the depression produced by the ligature on the neck was, as in that case, nowhere ecchymosed."

When the expert is called upon to examine a person supposed to have met his death by hanging, he should carefully examine every part of the body for evidence of external violence. If bruises, wounds or other evidence of injury be found, the surrounding objects should be examined with reference to their nature and position in order to determine whether or not the injuries found

might have been inflicted by them accidentally in committing suicide, or whether they point to homicide. In committing suicide by hanging the individual may place himself upon a chair, table, or some other elevation, adjust the cord, and fall in such a way as to bruise or lacerate his flesh, or even fracture a limb. All these possibilities should be taken into consideration in deciding whether or not death was due to hanging.

A closer study of the manner of death in hanging may be profitable. While closure of the trachea and death by mechanical asphyxiation are the objects sought and usually attained, there are certain complicating and modifying factors. When the noose is placed about the neck and the weight of the body, either wholly or in large part, put on the cord, death sooner or later results, and may be due to one of three causes acting individually or collectively. The noose incloses respiratory, circulatory and nervous connections and death may result from the interruption of the function of one or all of these. Undoubtedly in the great majority of hangings all of these connections suffer more or less and injury to each contributes to the final result. When the respiration is completely interrupted death within three minutes is certain, but in many instances of even legal hanging the passage of air is not wholly prevented. Complete arrest of the flow of blood through the carotids leads to immediate loss of consciousness, but when this is the only or chief injury done, resuscitation after relatively a long time is possible. Compression of the pneumogastric nerve may lead to immediate arrest of respiration, even in case the passage of air through the trachea is not mechanically interfered with; then, in legal execution by hanging, when the body is dropped some distance, the spinal cord is more or less injured, a vertebra may be fractured and indeed the head may be severed from the trunk.

That death may be caused by hanging without asphyxia has been demonstrated by men hanging themselves successfully while wearing tracheotomy tubes and with the cord placed above the incision in the trachea. Reineboth¹ has collected the earlier literature bearing on this subject, has reported a case and recorded his own experiments on animals. A man, who was wearing a tracheotomy tube on account of a tumor involving the trachea and esophagus, placed a cord above the tube and successfully hanged himself although he could touch the ground with his feet. Reineboth's animal experiments confirmed the earlier researches of Misuraca and established the fact that tracheotomy does not save animals from death by hanging but that the tracheotomized animal lives longer after suspension and that the appearance both *in vivo* and postmortem are not the same. The longest time that a normal rabbit lived after suspension was 3.3 minutes, while the longest time that a tracheotomized animal lived was 19.15 minutes. Bertelsmann² reports a case quite similar to the preceding. A woman who was wearing a tracheotomy tube was found dead fifteen minutes after she had been seen. Her knees touched the floor and her hands were folded across her bosom, so that she might easily have saved herself. She probably became immediately unconscious from the compression of the blood vessels.

Hofmann³ seems to have been the first to call attention to the influence of arterial compression in hanging on the sudden loss of consciousness. He stated that the sudden loss of consciousness and the more speedy death, compared with that due wholly to asphyxia, occurring in hanging, are caused by compression

of the blood vessels and injury to the vagus nerve. This statement was seriously questioned until apparently settled by the thorough researches of Haberda and Reimer⁴ whose conclusions are stated as follows.

1. The occlusion of both carotids in hanging, as claimed by Hofmann, cannot be denied.

2. In typical hanging both vertebral arteries are occluded. This interruption of the blood flow accounts for the sudden loss of consciousness.

3. There may also be suspension of the heart beat in diastole. Whether this is caused directly by injury to the vagus or reflexly through the laryngeal is left an open question.

4. This nervous effect, in cases of incomplete interruption of the blood flow, may hasten unconsciousness and delay asphyxia.

5. Brain pressure has no marked influence on the phenomena of death by hanging.

6. Traumatic injury to the laryngeal nerve probably has some effect in arresting respiratory movements, as suggested by Ignatowsky.

The third important factor in determining the phenomena of death from hanging is found in the injury done the vagus and its branches, especially the superior laryngeal, and its branches. Many years ago Czermak⁵ called attention to the fact that pressure on the vagus may markedly increase the length of the interval between heart beats. This finding has been repeatedly confirmed and if such an effect can be induced by pressure on the vagus with the finger, it is easy to believe that serious results may follow the greater and more violent pressure of a hangman's noose. The experimental evidence on injury to the trunk of the vagus in hanging has been somewhat contradictory. However, the studies of Placzek⁶ seem to show that the constriction of the noose has but little if any effect upon the vagus itself, because this nerve is protected by adjacent and superimposed muscles and that the effect of a noose is quite different from that of a finger nail that may be pressed forcibly upon one point. That the superior laryngeal is often injured and that this may and does contribute to the causation of death from hanging seems quite well established.

Usually in hanging the noose is placed above the thyroid and between this and the hyoid bone. With the cord in this location the base of the tongue is pressed against the posterior wall of the pharynx, the posterior nares are closed by the soft palate, and the apex of the larynx is brought upward and backward. In this way the passage of air may be wholly prevented. With the cord across the thyroid the occlusion of the windpipe may be only partial.

Haughton of Dublin, many years ago, investigated the mechanism of hanging and concluded that the most speedy and painless death is secured by placing the knot under the chin and allowing a fall of from ten to fourteen feet, inasmuch as by this method the spinal cord is fractured. Louis, after observing this method of legal execution, concluded that the executioner should give the body a violent twist as it falls, claiming that this movement fractures the odontoid process, dislocates the upper cervical vertebrae and results in instantaneous death.

Loss of consciousness in hanging occurs as soon as the noose is tightened by the weight of the body and the whole weight of the body is not required. The instantaneous loss of consciousness is shown by the fact that the suicide

never attempts to save himself after tightening the cord, although in many instances he might do so by straightening his legs or extending his arms. The same thing has been repeatedly demonstrated in accidental hanging. There are several cases on record where children in play have hung one another and if the victim throws a part or the whole of his weight against the cord he makes no attempt to save himself. Still more striking are some instances in which men in studying the sensations of asphyxia have carried the experiment beyond control. There are other instances in which there are strong reasons for believing that the suicide was seeking sympathy and not death. He provides himself with objects that he can easily reach with the intention of being found half dead, but when the cord tightens he loses consciousness and is no longer able to save himself from his folly. Hanging oneself and hanging a friend in play are dangerous experiments, because they are likely to become too realistic. Ziemke⁷ points out that unconsciousness in hanging is not due to lack of blood in the brain, but to lack of arterial blood, or of oxygen. Anemia of the brain, when found after death from hanging, exists before suspension. The compression of the arteries arrests the flow into the brain and when incomplete it may lead to venous engorgement.

While unconsciousness follows suspension instantaneously, death does not follow until after the lapse of some minutes. While there may be immediate arrest of the heart through the vagus center, this is only transitory and the heart beats begin again. The time that elapses between suspension and permanent arrest of the heart is variable. Usually the heart permanently ceases to beat within from five to sixteen minutes. In attempts at suicide the occlusion of the blood vessels is often incomplete and for this reason recovery may follow relatively long periods of suspension. The symptoms observed in those who have recovered from hanging are variable. There may be paralytic symptoms, especially of the bladder and bowel. Difficult respiration, bronchial catarrh, expectoration of blood and more or less protracted fever have been observed. The symptoms referable to the central nervous system, such as convulsions, sensation anomalies and hallucinatory delirium, have given rise to considerable discussion and the expression of marked differences of opinion. In some there are marked contortions, in others there are seizures resembling those of epilepsy, while in many there is extraordinary increase in reflex excitability. Some authorities hold that most, possibly all, of these manifestations are hysterical. It is worthy of note that in persons who have recovered from hanging there may be a retroactive amnesia, in which the victim may fail to remember not only everything connected with the hanging but events that happened before that time.

While in legal execution by hanging the body is dropped some distance, in suicide the body may have any position that man may voluntarily assume. In 261 cases, Tardieu found 168 with the feet touching the ground, 42 on their knees, 25 lying horizontally and 19 sitting. Brouardel has determined the weight necessary to cut off the flow of blood through the vessels of the neck and has found that two kilos compress the jugular veins, five close the carotids, fifteen shut the windpipe and thirty occlude the vertebral arteries. Besides the weight suspended, the nature of the cord is an important factor, a narrow cord being more effective than a broad one. Moreover, the presence of something,

such as a long beard, articles of clothing, or tumors of the neck, under the cord, have an effect.

It seems that some writers have overdrawn the unsightly appearance of the face of the hanged. As a rule the face of one who has come to his death by suspension does not differ from that of those who have died from other forms of violence. Occasionally there is marked cyanosis of the face, but Schmidt found this condition in only 24 out of 344 deaths from hanging and Reuter did not observe marked cyanosis once in 200 cases. Ziemke^s states that the description of the face of the hanged as given by Martin is a "phantasy picture." He also says that he has not seen unequal dilation of the pupil and the open lids more frequently in the hanged than in those dying from disease. Ecchymoses, from the size of a pin point to that of a pea, are sometimes, not always, found on the forehead, on the lids and in the conjunctivæ, less frequently in the skin of the neck and chest. Lochte saw these in only eight out of 80 deaths by hanging. Schmidt observed conjunctival ecchymoses in 133 out of 344 cases, and Martineck in 17 out of 184. While these ecchymoses are found sometimes in the hanged, they also are seen in deaths from other forms of violence. Ziemke found ecchymoses in the conjunctivæ and gums of a child that had been killed by a blow with a hammer on the forehead and then hung. Rupture of the ear drum, it seems, may result from hanging but is by no means frequent.

The location of postmortem ecchymoses on the body may be of service in the examination of the hanged, but ordinarily they do not differ in distribution from those observed in the dead from other causes. The fluid state of the blood leads to the ready appearance of areas of hypostatic congestion and since the hanged are usually soon cut down and given a horizontal position they are most frequently seen on the back. However, when the body remains suspended the hypostatic areas of redness are seen mostly, or exclusively, in the dependent parts, especially in the limbs. The exclusive location of these congested spots in the dependent portions of the body, especially if they remain in these locations after the body has been placed in the supine position, is stated by Ziemke to be proof that the body has been suspended at least eight hours, because after this time the ecchymosed blood does not change its position with changes in the posture of the body. The fluid blood soon after death gravitates to the lowest parts of the body and when the hanged man is on his knees the thighs may show large areas of ecchymoses, while these stains may be wholly lacking below the knees. In one instance Schmidt found that the downward flow of the blood was arrested by tight garters.

Much has been written about the occurrence of a sexual orgasm in death from hanging, because the penis has occasionally been found to be erect and the underclothing stained with seminal fluid. That the ejaculation of semen does occur in some instances cannot be denied, but that there is any pleasurable sensation connected with this act, there is not the slightest reason for believing. Death from asphyxia whether caused by mechanical means or by the administration of a poison that acts on the respiratory centers, is often accompanied by the ejaculation of semen. Turgescence of the sexual organs in both sexes after hanging is common.

The appearance and character of the groove or mark made by the cord about the neck are worthy of the closest study. It seems that everything long

enough and sufficiently pliable has been tried by the suicide who has chosen hanging as his mode of exit. Rope, cord of every kind, leather straps, wire, suspenders, garters, cravats, handkerchiefs, towels, sheets, twisted straw, bark and pliable limbs of trees are some of the things which the ingenuity of the suicide has converted into a hangman's noose. It is quite evident that with such a variety of cords the marks and grooves must differ greatly in appearance. Then, there are differences in the adjustment of the noose that affect the shape, size and course of the line of strangulation. The more cautious suicide prepares a slip noose with the cord going all around his neck and so arranged that it tightens down when he throws his weight on it. The less skillful man prepares an immovable loop, which he places under his chin and then throws himself forward, steps from a chair, or bends his knees, or in a sitting, squatting or even in a horizontal position presses against the cord until the circulation to the brain is arrested, which, as has been seen, may be instantaneous, when he loses consciousness and is no longer able to extricate himself or to call for help.

When a broad piece of cloth as a folded towel or sheet, or a broad band of any material, is employed there may be merely a slight discoloration along the path of application and in rare instances this may be so superficial, especially when the body is removed from the noose soon after death, that it wholly disappears before the legal inspection is made. Strassmann reports such an instance: A man hung himself with a silk scarf, the body was taken down after fifteen minutes, and at the obduction no trace of the mark could be detected. Maschka reports a similar instance in which every visible sign of the local effect of the noose disappeared within twenty-four hours. It is said that when the strangulation mark is slight and the body is placed in water the discoloration may shortly disappear. This is one extreme while at the other a wire cord may cut into the soft tissues and when the body is left suspended for some hours it may sink into the vertebræ. Even when a broad band has been employed it may be that a seam in it or one edge may act like a thin cord and make a deep groove, or there may be a broad mark and a deep narrow groove. It is customary to speak of two kinds of strangulation marks. One is pale in color and soft in consistency. It is due to the gradual pressure of a broad, soft band, which slowly removes the blood from the part directly pressed upon while just outside of this area, both above and below are lines of congestion, which may become more deeply colored from hypostasis. In these instances the line of compression is generally gray or grayish-blue, although when superficially observed it may appear quite pale. The second kind of strangulation line is brown in color, dry and hard. This was once called a mumification line and was believed to occur only when the epidermis was destroyed by the constricting cord, but the microscopical studies of Schulz have shown that it is not so much due to abrasion of the epidermis, as to the removal of the fluids of the tissue by greater compression. This author distinguishes between a leather-like and a parchment-like line, the latter differing from the former in the greater intensity of the pressure and the consequent greater drying out of the tissue. Both become darker, drier and harder on exposure to the air, due to the evaporation of fluid. The dry, hard, dark lines most frequently result from strong, narrow cords, such as small ropes and especially from binding twine.

It was once supposed that sugillation in the strangulation line always oc-

curs when death results from hanging and that it never occurs in a suspended corpse, but more thorough investigation has shown that it is the exception, though it may occur in death from hanging, and that it may also be found in a suspended corpse; therefore, sugillation alone is no proof that the hanging was done during life.

It was suggested by Neyding⁹ that the microscopical demonstration of pinpoint hemorrhages in the compressed tissue might be taken as an evidence that death was caused by hanging, but others have shown that these may occur in a suspended corpse. Greater stress should be placed upon the macroscopic vascular congestion along the edges of the strangulation line, but this must not be confounded with postmortem hypostasis.

When the cord is brought two or more times about the neck there will be multiple strangulation lines with intermediate crests and sugillation in the latter may be marked. It is easily evident that the blood is forced into these crests by the constrictions above and below and this has been taken as evidence that death was due to hanging and taken with other things it undoubtedly has its value, but alone it is not convincing proof because the coloration may be due to postmortem diffusion, and when observed in a hypostatic area the livid crest is without value in determining the manner of death. However, Bockavius¹⁰ thinks that the character and localization of the vascular hyperemia and the nature of the extravasations both along the strangulation line and in the crests, when these exist, may be of diagnostic value when studied microscopically. He thinks that when these hyperemias and extravasations occur during life they are due to an active vascular congestion and that this may be recognized microscopically by the fullness of the arterioles, which in death are empty, and in death the only congestion possible is venous. This is a fine distinction and in the hands of an expert it may be of great service, but without unusual experience it might be difficult to distinguish between the smallest veins and arteries, especially when they are filled with blood.

Rarely blisters filled with serum more or less colored with blood are found along the strangulation line and in the crests, when these exist. It would seem that these could hardly occur in a suspended corpse, but Schulz says that they may. At any rate they are so infrequently observed in death from hanging that but little diagnostic value can be attached to them.

In the study of strangulation marks and grooves due to hanging some most unexpected questions have arisen. It has already been seen that these marks may be so slight that they are not easily recognized and that they may soon wholly disappear. On the other hand, it has been found that in rare instances lines and grooves resembling those caused by the noose have been found to be due to other causes. Ziemke states that the creases in the necks of fat (and dirty) children may show marks closely resembling those due to a noose, and Maschka relates the case of a little girl who had a narrow band of some inflammable material about her neck. This caught fire and quickly burning there was left about the neck a line similar to that of a noose. Lesser tells of an epileptic that was found in the morning with his face on the floor and his legs on the bed and that the collar of his nightshirt had produced a strangulation line about his neck, but Ziemke suggests that possibly this man actually died from hanging.

Examination of the viscera after death by hanging shows nothing that is

strictly characteristic. When all the vessels of the neck are completely occluded by the cord, blood can neither enter nor leave the brain. Profuse hemorrhage in the cranial cavity after death by hanging is rare and probably occurs only when the vessels are diseased and consequently are easily ruptured. However, it is not strange that sudden arrest of the circulation in the brain may lead to moderate engorgement, which is likely to be greater when the arterial flow is only partially obstructed. Instances of paralysis among those who have attempted suicide by hanging and have been found in time to save life have been reported. When the brain is found to be anemic, as is sometimes the case, it must have been in this condition before the tightening of the cord, because, as has been seen, the jugular veins are easily compressed. Retinal and retrobulbar hemorrhages seem to be very rare, but have been observed.

Severe pulmonary hemorrhages are rare, but have been seen, especially in the tubercular. The quantity of blood in the lungs after death from hanging is very variable. It is probably largely determined by the coincidence of the drop with expiration or with inspiration. In the former instance the pulmonary vessels contain less blood than they do in the latter. Lochte¹¹ thinks that the degree of compression on the vessels of the neck has much to do with the volume of blood in the lungs. In incomplete closure of the vessels the duration of asphyxia is lengthened and passive hyperemia of the lungs and other organs may take place.

Edema of the lungs, generally confined to one lobe and sometimes to a part of a lobe and resulting from a terminal stasis, has been observed. Lochte found it in 23 out of 71 cases of suicide by hanging and Strassmann has seen it especially when the knot lies over the throat. He thinks that cyanosis of the face and edema of the lungs occur only when the occlusion of the vessels is incomplete but that they do not always occur when this condition prevails.

Sometimes hemorrhages are found in the mucous membrane of the alimentary canal. Ziemke is of the opinion that in the majority of instances they result from hypostasis, but that in some there is rupture of the smaller blood vessels during the convulsions of asphyxia. The writer wishes to suggest that punctiform hemorrhages in the mucous membrane of the stomach occur frequently in the convulsions of asphyxia whether the failure of respiration is due to mechanical pressure on the larynx or to the administration of a poison that acts upon the respiratory center. Hemorrhage into the stomach, which could not be due to hypostasis, has been reported by such careful observers as Tourdes, Lesser and Hofmann after death by hanging. Tourdes observed gastric hemorrhage in 31 out of 60 cases of death from hanging.

Occasionally food from the stomach has been found in the larynx and more rarely in the fine bronchi. In the former instance it is likely that the handling of the body in taking it down may account for the bits of food found in the larynx but they could be carried to the smaller bronchi only by inspiration and must be transported before the windpipe is occluded.

Ziemke gives the following directions for the dissection and examination of the neck after death from hanging: After the separation of the skin from the fascia and muscles, the muscoli omo-hyoidei should be divided in the middle on the inner border of the muscoli sterno-cleido-hyoidei and dissected out, then the sterno-cleido-mastoidei should be separated from attachment to the

clavicle and the sterno-hyoidei from the hyoid bone, and the deeper lying sterno-thyroidei and thyreo-hyoidei should be severed, dissected out and turned back.

Hemorrhage of the deeper soft tissues of the neck after death by hanging is not frequent. Martineck observed this only ten times out of 184 cases and Schmidt eleven times in 344. It is more frequent after legal execution than in suicide owing to the greater violence with which the former is done. Marked hemorrhage in or between the muscles of the neck is regarded as conclusive proof that the injury was inflicted during life. Laceration of the muscles of the neck is also rare in suicide. The most important findings in dissection of the neck consist of fractures of the cartilages of the larynx, of the thyroid and of the hyoid bone. Fracture of one or more of these structures occurs probably in the majority of cases of death from hanging. Fracture or displacement of cervical vertebræ may occur when the drop is great as it is in legal execution, but is infrequent in suicide. Rupture of the intima of one or both carotids may happen and there is one case reported from the Berlin forensic institute in which there was rupture of the intima of the jugular vein.

The question whether death was self-inflicted or not is an important one and deserves further study. Leaving out of consideration all instances of legal execution and of mob violence in which as a rule there is no room for doubt, the decision between suicide and murder will be discussed. In the first place it should be stated that hanging is a common form of suicide and a rare one for murder, so that the presumption is in favor of the former, but this does not relieve the one who investigates the case from the necessity of exercising the greatest care. The fact that hanging offers a speedy, painless and noiseless death and that the means of carrying it out are always at hand probably explains its frequent selection by the suicide. On the other hand it is not easy for one person to hang another if the victim is aware of the intention of his assailant, unless the latter is much superior in strength to the former. Children and old and feeble people may be hung, but this is not, as has been stated, a frequent resort of the murderer.

When a body is found freely suspended with nothing at hand whereby such a position could have been reached, help in the execution of the deed must be evident.

The suicide often leaves some record of his intention and his clothing and his belongings should be carefully searched for such evidence. If a written statement be found the question becomes one of identification of the handwriting. Bodily infirmities in the form of incurable disease, involving continuous suffering, financial losses, family disagreement and melancholia from any cause are some of the conditions that lead to suicide.

The cord used in the suspension should be examined. Where did it come from, to whom did it belong or in whose possession was it, are questions that arise naturally. As a rule the suicide selects something that will not cut deep with the idea that it will cause less pain. For like reason the suicide, as a rule to which, however, there are exceptions, does not provide for a long drop.

The fact that the victim is a child will be taken into consideration but children as young as eight or ten years have been known to commit suicide in this way. The presence of wounds on the body must be investigated. These do not, however, preclude suicide and many cases are recorded in which at-

tempts to destroy one's own life have been repeatedly made and not proving successful hanging has been resorted to. In some instances attempts have been made to cut the throat or to open an artery, but courage or skill being lacking, the noose has been employed; in others, poison has been taken.

There are a few cases, the number is not large, in which people have been killed in some other manner and then the dead body has been suspended by the murderer in order to present the appearance of suicide. Vrolik tells of a sailor who was stabbed through the heart. His murderer washed the body, dressed it in a clean shirt and suspended it. Maschka reports the finding of a suspended corpse the skull of which had been fractured by a blow. Gross tells of a man who drove a long, sharp needle through the chest wall, under the breast of his wife, into her heart and then suspended her body. If one has been strangled to death and then suspended it might be quite difficult to determine the cause of death unless the imprint of fingers on the throat be found. Lesser reports an instance of a man who, while sitting on a stool, was approached from behind, a cord was thrown about his neck and twisted. He fell from the stool in an unconscious state and after his death his body was suspended by his assailant.

Ziemke¹² gives the following as proofs that hanging was the cause of death:

1. Profuse effusion of blood under the strangulation groove when shown not to be due to hypostasis.

2. Marked vascular congestion along the edges, especially the lower edge of the strangulation groove, which is found on macroscopic examination to consist of a network of distended vessels, when this is shown with certainty not to be due to hypostasis.

3. A large number of isolated blood vessels that pass through the groove of strangulation are filled with blackish-red blood and are brought out more distinctly by treatment with alcohol-xylol.

4. A red intermediate crest, if it is broad and remains prominent and firm after the removal of the cord, lies in paler skin and is found on microscopic examination to consist of congested vessels and hemorrhages into the cutis and subcutaneous fat.

5. Profuse hemorrhages in and between the muscles of the throat and lacerations of the muscles and fractures of the larynx with profuse blood extravasations, when shown not to be due to hypostasis.

6. Lacerations of the intima of the carotids with extravasated edges and hemorrhages into the sheath of the vessel.

7. Marked cyanosis of the face, with numerous small hemorrhages in the skin, which extend down to the groove and stand out in contrast with the paler surface of the rest of the body.

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⁷Casper-Liman's Handbuch, 1907.

⁸Casper-Liman's Handbuch, 1907.

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¹⁰Casper-Liman's Handbuch.

¹¹Viertelj. f. gerichtl. Med. 1901.

¹²Casper-Liman's Handbuch, 1907.



Fig. 1.—To show the contrast between the constricted vessels still connected with the vaso-constrictor center (left ear) and the control dilated vessels that have been disconnected from that center (right ear). Drawing made late in the experiment when the animal was apparently in a state of deep shock. The strong vaso-constriction in the left ear was replaced by a wide dilatation as soon as the connection of this ear with the vaso-motor center was severed. (See Protocol V.)

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ORIGINAL ARTICLES

ON THE CONDITION OF THE VASO-CONSTRICTOR CENTER DURING THE DEVELOPMENT OF SHOCK*

BY MAJOR G. SEELIG, M.D., AND DON R. JOSEPH, M.D., ST. LOUIS, MO.

SO many reviews of the literature on surgical shock have appeared that we deem it unnecessary to enter here into such a discussion. The term is in itself too generic and although we make no attempt to define it, we use the term surgical shock to cover that compromise of the animal which results from operative trauma and which is analogous to the compromise that occasionally follows surgical therapeutic procedures on man. Our interest is centered in the view, which has been accepted too generally and upon insufficient evidence, that in surgical shock the *primary* cause of all the other symptoms is a preceding paralysis of the vaso-motor center. Since Porter first attempted to show that the vaso-constrictor center is still in a state of pronounced functional activity after a shock level of blood pressure has been reached, there have been attempts made by several investigators to bring forward evidence bearing upon this point.¹

PROBLEM.

Our problem was essentially this: Can we obtain any conclusive evidence that, during the development of shock, the vaso-constrictor center at any time loses its activity? If we could show that the center loses its activity very early, it would be reasonable to believe that some, if not many of the other symptoms of shock, are secondary to this failure. If, on the other hand, indisputable

*From the Department of Physiology of St. Louis University School of Medicine.

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Also a demonstration of the results herein described was given before the Amer. Physiol. Society at its meeting in St. Louis, in Dec., 1914.

¹Porter, W. T., and Quinby, W. C.: Am. Jour. Physiol., 1914, 10, xii.

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evidence could be obtained that the activity of the vaso-constrictor center persists for a long time and even after many of the other manifestations of shock are present, we could then say definitely that shock is not the result of a primary breakdown of the vaso-motor center but must be due to some other cause or causes.

Our first requirement was a region of blood vessels, the calibre of which could be observed without any operative interference. Second, a region the observations upon which could be controlled by comparing it to its denervated mate upon the other side of the midline, in the same animal.

The retinae of albino rabbits are non-pigmented and the retinal vessels stand out distinctly. In our early experiments, we tried to denervate the vessels of one retina and after producing shock in the animal, to observe and compare the size of the vessels in the two retinae. Theoretically this method should be satisfactory, but for practical reasons it was finally discontinued. We need mention here only two of these reasons. First, the origin and course of the vaso-motor supply to the retinal vessels is not perfectly understood; consequently, it might be claimed that the retinal vessels of the supposedly denervated side were not entirely isolated from connection with the vaso-motor center, in which case we should have no control vessels. Second, it is necessary to depend upon the judgment of the ophthalmologist for a comparison of the vessels in the two eyes, thus introducing too many opportunities for error and furnishing us no chance to check our own results by direct observation.

We finally selected the ear vessels of white rabbits. This region seems almost ideal for our purpose. First of all, the vessels can be seen distinctly by anybody. This means that a comparison of the ear vessels can be made by any number of individuals, either trained or untrained. Secondly, the vessels are subjected to no direct operative interference at any time. Thirdly, the course of the vaso-constrictor nerve fibers to the ear vessels has been well worked out² and we can be sure therefore that the vessels of the control ear have really been disconnected from the medullary center.

The following is the plan by which we attacked the problem: If the vaso-constrictor nerves to one ear are cut we remove all influence of the vaso-constrictor center over the vessels of that ear. The denervated vessels might then be expected to react more or less passively to changes of pressure within them. If the heart were maintaining a high blood pressure these vessels should become passively dilated. If, on the other hand, the blood pressure were low they should be correspondingly less dilated. In other words, the control vessels of the denervated ear should react passively no matter what the pressure within them. Why do we need such a set of control vessels? Suppose we reduce an animal to a state of shock and find that ear vessels connected with the vaso-constrictor center are empty. This emptiness may be either an active or a passive one. That is, it may be caused by a stream of vaso-constrictor impulses flowing out from the center in the medulla and maintaining in the constricted vessels a tonus sufficiently active to force out the blood, or it may be due to a passive drainage of the blood from these vessels into the great splanchnic reservoir. If we denervate the vessels of one ear, the vessels of that ear—if they respond passively

²Meltzer and Meltzer: *Am. Jour. Physiol.*, 1903, ix, 57.

to pressure changes within them—will tell us whether the constriction present in the normal ear is an active or a passive one. Especially is this true if we can, by suitable means, raise temporarily the blood pressure from its low shock level. The denervated vessels should passively dilate in response to this elevation of pressure. The normal ear vessels, if they are receiving no vaso-constrictor impulses should also dilate; if, however, they resist the rise in pressure and remain constricted, we are justified in concluding that the constriction is the result of vaso-constrictor impulses arising outside the vessels, presumably in the medulla.

Furthermore, the ability of the vessels of the normal ear to remain constricted under the temporarily increased blood pressure should be an index of the degree of tonus being maintained by the center in those vessels. For example, if the constriction persists in the normally innervated vessels after the blood pressure has risen to a high point, we must conclude that the center is maintaining a high degree of tonus.

The question of the degree of passive response obtainable from denervated vessels at different intervals after denervation will be discussed a little later.

EXPERIMENTAL METHOD.

As already stated, white rabbits were used exclusively. The vessels of one ear were denervated by resecting and severing, under ether anesthesia, the auricularis magnus nerve at the base of the pinna and removing the superior cervical sympathetic ganglion on the same side. In no case was the animal reduced to a state of shock immediately following the denervation of the ear. At least 20 hours elapsed between the two operations and in some cases this interval was a number of days in length. The purpose of this delay was to avoid the effects upon the denervated vessels of immediate stimulation of vaso-dilator or vaso-constrictor nerve fibers consequent upon the operative procedures at the time of the denervation. After this interval, the animal was again etherized and reduced to a state of shock. In most cases it was not necessary, nor indeed advisable, to continue the etherization for more than $\frac{3}{4}$ to 1 hour, for at that time most animals showed only a slight reaction, if any, to sensory stimulation. Tracheotomy was performed and the abdomen opened wide by a long median incision. In a majority of the experiments the adrenals were carefully removed at once upon opening the abdomen. This was done on the assumption that adrenalin thrown into the blood stream during the experiment might possibly interfere with a passive dilatation of the vessels of the denervated ear, and at the same time favor constriction in the vessels of the normal ear, a constriction which would be of peripheral rather than of central origin. Also it was thought that the removal of the adrenals might hasten somewhat the fall of the blood pressure. We are unable to draw any definite conclusions as regards either of the above possibilities since we made no systematic comparison between animals with intact and with excised adrenals. At any rate, the removal of the adrenals, practiced in most of the experiments, with the attendant manipulation of and traction upon the viscera and sympathetic nervous structures may be considered one of the means used to induce shock.

Next the abdominal aorta was exposed for a short distance between the origin of the celiac artery and the diaphragm, and a loose ligature passed around it to facilitate the application of a clamp when desirable. Clamping the aorta at this point was the method used to raise temporarily the blood pressure, in the part of the animal anterior to the diaphragm, when the general blood pressure had fallen to a low level. The reason for raising the blood pressure at this time has already been stated. Tracings of the blood pressure were taken from a mercury manometer connected with a cannula in one axillary artery. As an index to the degree of shock we have relied largely upon the state of the blood pressure; we did not confine ourselves, however, to observations upon this one symptom of shock. The state of the reflexes was used as was also the response to sensory stimulation applied to various regions. We continued the experiment not only until the blood pressure had fallen to a low level but until the reflexes became sluggish or were practically absent, and there was little, if any, response to handling of the abdominal viscera or parietal peritoneum. The rabbit seems to sink to this condition much more readily than do most dogs.

As a second and final test of whether the constriction in the normally innervated vessels was an active or a passive one, we denervated, late in the experiment, the vessels of the normal ear. This was done in some cases by cutting the nerves, in others by abolishing the nerve conductivity through direct application of an ether soaked pledget of cotton to the nerve trunk, or by freezing the nerve at one point with an ethyl chloride spray. We found that, for practical purposes, severing the conductivity of the cervical sympathetic trunk was sufficient to bring out the effect in the "normal" ear vessels and therefore in most cases we did not cut the auricularis magnus nerve. Another reason why we usually left the auricularis magnus intact was that we wished to disturb the animal as little as possible. (The cervical sympathetic is the more easily obtainable of the two nerves that carry a vaso-motor supply to the ears.)

In a few experiments the ears of the animals were photographed during the course of the experiment. For this purpose we used light transmitted through the ears to the camera from an electric arc. In one case a water color drawing was made from life toward the end of the experiment.

EXPERIMENTAL RESULTS.

Twenty-five experiments were performed in this series. The interval between denervation of the blood vessels of one ear and the production of shock varied. In eleven experiments this interval was 20 to 28 hours; in two experiments it was 2 days; in five experiments it was 3 days; in two experiments 4 days; in two experiments 6 days; and finally one experiment each was performed in which the interval was 7, 9 and 12 days respectively. As has already been stated, this interval was allowed in order to secure a proper set of control vessels. It concerns in no way the vessels of the normal ear directly.

On the Suitability of "1," "2," "3" (and more) Day Rabbits for this Kind of Test.

The term "one," "two," or "three" day rabbit will refer to the length of the interval which elapsed between the denervation of the blood vessels of the control ear and the execution of the shock experiment.

We found early in the series of experiments, that the denervated vessels of "one day" rabbits responded best. That is, their response was most nearly a passive one. If a longer time elapsed the denervated vessels showed an increasing tendency, with the increase in the length of the interval, to constrict and a decreasing tendency to react passively to pressure changes within. The following figures will serve to make this point clear.

In 11 experiments we used "one day" rabbits. The denervated vessels invariably showed good passive responses to pressure changes. In 5 experiments we used "three day" rabbits. In four of these the passive response of the denervated vessels was good, but in the fifth scarcely any passive response could be obtained. Two "four day" rabbits were used. One of them failed to show a passive response. Of the two "six day" rabbits used one failed to respond passively. Finally, one experiment each was performed upon "seven day," "nine day," and "twelve day" rabbits respectively. The "seven day" rabbit gave a good passive response but the remaining two showed practically no passive reaction whatever. To summarize these results we may say that in every case when used within one day after denervation (11 cases), the passive reaction of the vessels was good; that they failed to react well in one out of five "three day" rabbits, while in seven experiments upon rabbits of "four days" or over, the failure to respond passively was seen in four, that is, in about 57 per cent.

What is the significance of these results? It is this. We require, for our demonstration, vessels which respond passively to pressure changes within them in order that, by comparison, we may determine the state of tonus being maintained by the vaso-motor center in the vessels with which it is still connected. The results stated above show that to obtain this passive response in the control vessels, "one day" rabbits are best; that, while it is sometimes possible to obtain a passive response, even as late as seven days after denervation, still in many cases the vessels have developed by that time too much autonomy to serve as passive controls.

The above observation agrees with statements in the literature³ to the effect that the dilatation of the vessels of the denervated ear gradually decreases and finally may even disappear. The time necessary for the latter to develop is stated by Meltzer, however, to be a matter of weeks.

It is stated by Bayliss⁴ that the arterial wall reacts to an increase in arterial pressure by constricting. If this statement be true, can we use the denervated ear vessels as passive controls? Anrep,⁵ working later upon the same subject in Bayliss' laboratory, concluded that "a local form of reaction of the blood vessel wall to changes of tension is as yet unproven." Even though Bayliss' original interpretation were correct, namely, that if the pressure within an artery is increased, the artery responds by contracting, or, that if the pressure falls the arterial wall relaxes, his results would not necessarily be in conflict with our findings. This is true for the following reasons:

Bayliss does not say to what degree the arteries became constricted and there is certainly no evidence that they responded to the increased pressure with a strong constriction. It may indeed be true that the arteries and arterioles in

³Meltzer and Meltzer: *Am. Jour. Physiol.*, 1903, ix, pp. 62 and 159.

⁴*Jour. Physiol.*, 1902, 28, 220.

⁵*Jour. Physiol.*, 1912, 45, 318.

the denervated ears of our animals were not as much dilated as they would have been had they not possessed any such reaction to internal pressure. And especially may this be true of the denervated vessels a number of days after the denervation when they show a definitely greater tendency to remain constricted. That these vessels were more dilated in our experiments after raising the blood pressure than they were before, however, cannot be questioned. In many cases the dilatation was easily visible across the entire width of the room and seemed to be practically maximal.

Again, the constriction described by Bayliss was apparently of short duration. He does not give, in all cases, the full tracing, but in one that he does give (Fig. 7, p. 226) the duration of the constriction was about one minute. In some other cases it was apparently somewhat longer. In our experiments the high blood pressure was usually maintained for from 3 to 5 minutes and sometimes (see protocols below) even longer. A transient tendency to constrict would, therefore, have disappeared in all probability before we removed the aortic clamp. Moreover, in our experiments, the increase in blood pressure developed slowly and not suddenly as was the case in Bayliss' experiments, for the heart does not build up a high blood pressure in the head region instantly upon clamping the aorta. Since usually the efficiency of a stimulating agent varies directly with the suddenness of its application, perhaps the tendency of the vessels to react when the increase in internal pressure is developed slowly is less than when it is rapid.

When all these factors are considered it can be seen that there is not necessarily a conflict between the results obtained by Bayliss and those here described. Perhaps the most conclusive evidence that the denervated vessels do dilate when subjected to a high blood pressure is furnished by the well-known fact that after cutting the cervical sympathetic nerve in the rabbit (as in the Claude Bernard experiment) the ear vessels on the corresponding side become widely dilated. This result obtains in an otherwise normal animal and therefore in one with a high blood pressure. It must be admitted, therefore, that at least to the extent to which these vessels dilate under high pressure and empty themselves when the pressure falls they may be used as passive controls for the blood vessels still connected with the center in the medulla.

RESULTS OF THE SHOCK EXPERIMENTS.

There was a remarkable constancy in the behavior of the vessels of the normally innervated ear. The following protocols are typical of the results obtained:

PROTOCOL I.

Experiment 14. May 15, 1914. One Day Rabbit. White Male. 1700 grm. Yesterday at 4:00 P. M. denervated the right ear.

- | | |
|------------|--|
| 3:00 P. M. | Began operation. Before operation vessels of normal ear well constricted; those of operated ear fairly well dilated. After tying down the animal, vessels of both ears dilated. |
| 3:56 | Operation completed. It consisted of tracheotomy, wide opening of abdomen, removal of adrenals and freeing of aorta just below diaphragm. Canula inserted in left axillary artery for blood pressure record. |
| 4:00 | Blood pressure 32 mm. Hg. Lid reflex fair. Denervated ear moderately dilated. Normal ear vessels slightly smaller. |

4:09½	Clamped aorta. Blood pressure began rising rapidly.
4:11	Vessels denervated ear widely dilated. Normal ear moderately constricted.
4:13	Blood pressure 94 mm. Hg. Lid reflex active.
4:14	Clamp off aorta.
4:15	Denervated ear still moderately dilated. Normal ear pale; vessels strongly constricted. Blood pressure 30 mm. Hg.
4:25	Aorta clamped again. Blood pressure rapidly rose to 87 mm. Hg.
4:26	Denervated ear widely dilated. Normal ear blanched; bloodless.
4:35-40	Cut the auricularis magnus and removed superior cervical ganglion on left side—that is for normal ear. "Normal" ear vessels became maximally dilated. Aorta is still clamped and blood pressure high.
4:45	Clamp removed from aorta. Blood pressure fell at once to 22 mm. Hg.
4:47	Vessels both ears same size (moderately dilated).
4:48	No lid reflex. Blood pressure 22 mm. Hg.
4:49	Clamped aorta.
4:52	Blood pressure 88 mm. Hg. Slight lid reflex. Vessels both ears practically maximally dilated. Clamp off aorta.
4:57	Blood pressure 36 mm. Hg. Slight lid reflex. Vessels of both ears moderately dilated.
4:57½	Clamped aorta again.
4:59	Blood pressure 100 mm. Hg. Vessels both ears maximally dilated.
5:00	Clamp off aorta.
5:08	Animal killed.

In this experiment the blood pressure had fallen within one hour after the beginning of the operation to 32 mm. Hg. pressure—that is to about one-third of the pressure normally present. At this pressure there was only a slight, though definite difference between the vessels of the two ears. When the aorta was clamped at 4:13 and the blood pressure in the head region rose quickly to 94 mm. Hg. a marked contrast appeared between the vessels of the two ears. The denervated vessels became widely distended with blood while the normally innervated vessels continued to be moderately constricted. When the aorta was unclamped the blood pressure fell quickly to a low level again—namely 30 mm. Hg. There was still a difference, though not a marked one, between the ears. At 4:25 the aorta was clamped and the blood pressure rose to 87 mm. Hg. This time the difference between the two ears was even more marked than after the previous clamping, for the protocol reads that this time the normal ear was "blanched and bloodless," whereas before it was described as being "moderately constricted." In other words, it might be thought that the vaso-motor center had not only not lost any of its grip upon the blood vessels during the past fifteen minutes, but had actually increased its tonus. At this point in the experiment the final step in the test was made to determine whether the vessels of the normal ear were constricted because of the activity of the vaso-motor center, i. e., the vaso-motor nerves of the normal ear were quickly exposed and severed. The result was unmistakable, for the "normal" vessels quickly became maximally dilated. At no time thereafter were they again smaller than those that had been denervated the day before. Twice, later, the aorta was clamped and unclamped. The blood pressure rose from 22 to 88 mm. Hg. and from 36 to 100 mm. Hg., respectively. Each time the vessels of both ears dilated strongly with the rise in blood pressure and emptied somewhat when the fall came. That is, they both reacted in the *same* direction, in strong contrast to their reaction before the "normal" ear was denervated.

The following is an example of a one day (20 hours) rabbit in which, to-

ward the end of the experiment, the cervical sympathetic was frozen instead of cut, as in the former experiment:

PROTOCOL II.

Experiment 32. July 30, 1914. White Female Rabbit. 1550 gm.

Vessels of right ear denervated 20 hours before shock experiment.

- 8:10 A. M. Before disturbing animal in cage the vessels of normal ear were strongly constricted, while the denervated ear vessels were widely dilated.
- 8:15 Began operation. Method exactly same as in Experiment 14 (Protocol I), except that this time adrenals were not removed.
- 8:50 Operation completed. Vessels of both ears same size; moderately dilated.
- 9:06 Have been handling abdominal viscera for over ten minutes. Normal ear well constricted; denervated ear well dilated. A marked difference between them. Lid reflex good. Respiration 132.
- 9:43 Blood pressure 46 mm. Hg. Normal ear well constricted. Denervated ear moderately dilated.
- 9:44 Clamped aorta.
- 9:46 Blood pressure 72 mm. Hg. Normal ear still well constricted. Denervated ear vessels gorged with blood.
- 10:15 Clamp off aorta.
- 10:25 Again clamped aorta.
- 10:28 Normal ear vessels well constricted. Denervated vessels widely dilated.
- 10:30 Froze left cervical sympathetic nerve with ethyl chloride spray. Vessels of "normal" ear became quickly as widely dilated as those of denervated ear.
- 10:45 Clamp off aorta.
- 11:08 Blood pressure 30 mm. Hg. Respiration 44 per minute. Lid reflex sluggish. "Normal" vessels slightly dilated. Denervated vessels moderately dilated (i. e., somewhat wider than "normal" vessels).
- 11:09 Clamped aorta again. Blood pressure rose to 54 mm. Hg. Denervated ear vessels gorged. "Normal" ear (nerve frozen at 10:30) well dilated but definitely less so than denervated ear vessels.
- 11:10½ Clamp off aorta. Blood pressure fell at once to 23 mm. Hg. Lid reflex sluggish. Animal seems deep in shock. Allowed to die.

The results of this experiment are essentially the same as those of the previous one. The normal vessels remained constricted until the conductivity of the cervical sympathetic nerve on that side was temporarily abolished by freezing it with the ethyl chloride spray. They became then as widely dilated as the denervated vessels. The freezing was discontinued as soon as the vessels of the corresponding ear dilated. That the conductivity of the nerve had not been permanently abolished by the freezing is shown by the fact that 39 minutes later (11:09) when the aorta was again clamped the normal vessels did not dilate so strongly as the denervated vessels. Some permanent injury had evidently been done, however, for the normal vessels did not return again to a state of strong constriction. It will be noted that a good dilatation was obtained in this case by destroying the conductivity of only one of the nerves supplying the vessels of the normal ear.

In the following experiment the control ear was denervated three days before the shock experiment.

PROTOCOL III.

Experiment 34. Sept. 12, 1914. White Male Rabbit. 1250 gm. Left ear denervated three days ago.

- 8:10 A. M. Vessels of normal ear constricted. Those of denervated ear well dilated.
- 8:20 Operation began—tracheotomy, abdomen opened wide, aorta exposed just below diaphragm, canula in left axillary artery for blood pressure. Adrenals left intact.

9:33	Operation completed. Blood pressure 80 mm. Hg.
9:40	Lid reflex sluggish. Respiration 48, slow, efficient, regular. Normal ear vessels strongly constricted. Denervated vessels slightly dilated.
9:50	Clamped aorta.
9:54	Normal ear as before strongly constricted. Denervated vessels well dilated. A marked difference between them.
9:57	Clamp off aorta.
10:32	Blood pressure 53 mm. Hg.
10:32½	Clamped aorta. Blood pressure rose rapidly to 108 mm. Hg. Normal ear vessels bloodless as before. Denervated vessels gorged with blood. The difference can be seen across the room without difficulty.
10:37	Clamp removed from aorta. Blood pressure fell to 49 mm. Hg. Heart became irregular and slow. Lid reflex gone. Struggling, apparently dying. Clamped aorta again. Blood pressure rapidly rose to 119 mm. Hg.
10:42	Blood pressure 120 mm. Hg. Normal ear absolutely bloodless and blanched. Denervated vessels gorged with blood.
10:50	Clamp off aorta. Blood pressure fell to 48 mm. Hg.
10:52	Blood pressure very low. Lid reflex sluggish. Respiration 40 per minute, regular, efficient. Normal ear vessels blanched. Denervated ear pink, but vessels slightly constricted. No response to handling of viscera or stroking of parietal peritoneum. Aorta clamped.
10:52½	Ether applied to trunk of right sympathetic.
10:53½	"Normal" ear vessels widely dilated. Somewhat wider, in fact, than vessels of ear denervated three days ago.
10:55	Ether washed off nerve. Clamp off aorta.
11:05	Denervated vessels slightly dilated. "Normal" vessels somewhat smaller than control vessels. Aorta clamped.
11:08	"Normal" ear vessels definitely smaller than those of control ear, which are well dilated. Again applied ether to cervical sympathetic and normal vessels dilated as before.
	Experiment discontinued.

The first point to be noted in this experiment is that the control vessels reacted very satisfactorily to internal pressure changes. If they resisted at all the tendency toward dilatation when the blood pressure was raised by clamping the aorta, it was certainly not to any marked degree, for the word "gorged" described best the picture presented at that time. This engorgement did not confine itself to the main trunk vessels of the control ear. The whole ear was suffused to a marked extent. Inasmuch as three days elapsed between the denervation of this ear and the execution of the shock experiment, we believe it can hardly be claimed that this dilatation was in any sense due to a persistent stimulation of vaso-dilator nerve fibers that were injured three days before when the nerves were cut. The evidence in favor of this view is even more conclusive in the protocol which follows, for in that case *seven* days elapsed after cutting the nerves of the control ear before the shock experiment was carried out, yet the conduct of the vessels of the control ear was essentially the same as in the experiment under discussion.

The conduct of the vessels of the normal ear in this experiment is striking. Two and a half hours after the beginning of the experiment the blood pressure had fallen to 48-49 mm. Hg. (see Protocol at 10:37 and 10:50). When the aorta was clamped at 10:42 the blood pressure rose to 120 mm. Hg.—that is, to a high level for the normal rabbit. The general condition of the animal, at this stage of the experiment, was bad. The lid reflex was sluggish and sensory responses very poor or entirely absent. In spite of the state of the animal, generally, and in spite of the very high blood pressure, the normal ear

(10:42) remained "blanched and bloodless." Furthermore, the vessels remained persistently in a state of what seemed to be maximal constriction until the conductivity of the cervical sympathetic nerve on that side was abolished by the application of ether. Immediately thereafter the vaso-constriction of the normal ear was replaced by a marked dilatation, thus furnishing evidence that the preceding constriction was due to the active influence of the vaso-constrictor center. We wish to call attention to the following additional evidence that we are dealing here with an active vaso-motor center. The ether was washed off the cervical sympathetic as quickly as possible after the vessels of the "normal" ear dilated. This was done to prevent, if possible, a permanent abolition of the nerve conductivity. We had the satisfaction of seeing later a partial recovery of tonus by the vessels of the "normal" ear—that is, these vessels again became distinctly smaller than those of the control ear under the same pressure. That they did not again become strongly constricted, was due in all probability to some permanent injury produced by the ether. A second application of ether to the cervical sympathetic again brought about a strong dilatation of the "normal" vessels.

The fact that the vessels of the *normal* ear became slightly more dilated, after application of the ether to the cervical sympathetic, than those of the control (denervated) ear, illustrates the statement made above that vessels several days after denervation usually show a greater tendency to resist the dilating influence of internal pressure than do vessels that have just been denervated.

PROTOCOL IV.

Experiment 17. June 16, 1914. Seven Day Rabbit. White Male. 1850 gm.
Right ear denervated seven days before shock experiment.

8:14 A. M.	Vessels of denervated ear definitely, but only slightly, wider than those of normal ear.
8:15	Began operation. Tracheotomy. Abdomen opened wide, adrenals removed; canula in abdominal aorta at level of bifurcation into iliacs, for blood pressure tracing.
9:08	Began registering blood pressure. Blood pressure 42 mm. Hg. Normal ear vessels wide. Denervated ear vessels small.
9:25	Normal ear blanched. Denervated ear moderately constricted, but vessels definitely wider than normal ear vessels.
9:33	Clamped abdominal aorta above celiac. Normal ear remained blanched and bloodless. Denervated ear vessels fairly widely dilated and the whole ear bright pink in color. Could not register blood pressure while aorta was clamped.
9:38	Clamp off aorta.
9:39	Animal in very deep shock.
9:43	Blood pressure 30 mm. Hg. Lid reflex very sluggish. Normal ear bloodless. Denervated ear well suffused. Vessels moderately dilated.
9:47	Abdominal aorta clamped above celiac again.
9:49	Normal ear blanched as before. Denervated ear vessels almost maximally dilated. The difference between the ears is very striking.
9:54	Clamp off aorta.
10:05	Blood pressure 24 mm. Hg. Lid reflex very sluggish. Respiration regular, shallow, 52 per minute. Normal ear bloodless as before. Denervated ear moderately dilated and suffused.
10:10	Removed left superior cervical ganglion and clamped aorta above celiac.
10:12	Normal ear vessels (just denervated) almost maximally dilated. Wider than the other ear. Denervated ear vessels (former denervation) moderately well dilated.

10:28	Ears same as 10:12. Respiration 24 per minute. Lid reflex sluggish. Heart feeble and slow.
10:30	Clamp off aorta. Struggling. Blood pressure 15 mm. Hg.
10:34	Animal nearly dead. Blood pressure falling gradually. Killed.

As regards the conduct of the vessels of the normal ear the experiment differed in no essential respect from the experiments already described. Before, during and immediately after the operation, these vessels showed evidences of the usual varying intensity of vaso-constrictor tonus (8:14—moderate constriction; 9:08—wide dilatation; 9:25—blanched); later in the experiment, when the blood pressure became very low, they showed no relaxation at any time. Whether the aorta was clamped or not, the normal ear vessels remained practically maximally constricted.

This contrast between the vaso-motor reactions obtained in the early and in the late stages of the experiment was seen so frequently that we may say it was practically a constant phenomenon. The vessels of the normal ear were usually in a state of moderate constriction before the animal was disturbed in the cage and practically always became widely dilated in consequence of the handling, tying down and operating—in short, before the experiment began and during its early stages they were subject to varying degrees of contraction and dilatation. In the late stages, however, there was almost never a variation from a state of vaso-constrictor tonus sufficient to keep the normally innervated vessels strongly contracted. This may be interpreted to mean that early in the experiment, when the general blood pressure control by the vaso-motor center is ample, either a vaso-constrictor or a vaso-dilator response may be made by the center to a change in the environment; that later in the experiment, however, when the blood pressure has shown a strong tendency to fall, the vaso-constrictor center marshals all its forces and only one type of response is seen—namely a strong constriction; that, moreover, this constrictor influence is maintained through a period when other functions show marked signs of giving way.

Again, in this experiment, we see that the “normal” vessels became strongly dilated as soon as the cervical sympathetic path was interfered with. The chief reason for presenting this protocol is that the control vessels, seven days after denervation, reacted in the same sense as did those of the so-called “one day” rabbits. It is surely reasonable to conclude that the dilatation seen in this case in the denervated ear, after clamping the aorta, was due to mechanical distention of the walls caused by the pressure of the fluid within, and not to any vaso-dilator influence of nervous origin. We regard this as a point of vital importance. If it be proven that the denervated vessels respond to increased pressure by a distention, then the normal vessels should, if left to themselves, react in the same way. If they react in an opposite sense it must be due to some outside influence. We believe we have shown by the experiments here described that there is an outside influence and that it can be removed by severing the connection between the vaso-constrictor center and the vessels. Moreover, we believe the foregoing evidence warrants us in concluding that the vaso-constrictor center is not the first of the vital centers to fail. In fact, there is some warrant for believing that at a time when other functions have yielded definitely, the vaso-constrictor center still shows marked activity.

Again in this protocol we find an illustration of a statement made in the

early part of the paper, namely—that if several days elapse after denervation, the denervated vessels show a considerable tendency to establish autonomy. This is evident throughout the experiment.

There is one other fact to be noted in this protocol which was noted also in a number of other animals experimented upon several days after denervation of the ear. The denervated vessels showed a greater tendency to contract at the beginning of the experiment than during the latter part. We do not wish to enter at this time upon an exhaustive discussion of the possible reasons for this apparent paradox. It might be said in line with Bayliss' experiments, that in the beginning the stronger tendency to constrict was the impulse furnished the muscular wall of the blood vessel by the high blood pressure;



Fig. 2.—The rabbit is in shock. The left ear (constricted vessels) is in connection with vaso-constrictor center. The right ear (dilated vessels) has been cut off from central control. (See Protocol V.)

and that as the blood pressure fell, during the course of development of shock, this tendency also diminished and the vessels dilated. Against this view stands the fact observed in several instances that the vessels dilated late in the experiment more than in the beginning, even if the blood pressure, by clamping the aorta, was raised to from 70 to 90 per cent of the normal level. If the vessels were stimulated to constriction by the high blood pressure in the beginning, they ought to respond to pressure in the same way later on. Since we plan to investigate further the pressure reactions of blood vessels, we will leave a further discussion of this point until a future time.

The protocols already presented are representative of the results obtained

in by far the majority of the experiments. We may designate as the type experiment, one in which the development of shock was a gradually progressive phenomenon that could be represented roughly by a straight line with one end placed lower than the other. In some cases the descent into a condition of shock was relatively rapid; in others very slow. The former of these could be represented by a line indicating a steep slope; the latter by a longer line, having a much more gradual slope downward. In both of these the vaso-motor center was strongly resistant. So far as one could judge from the vessels of the normal ear there was no time during the experiment, unless it were at the very end, i. e., just before death, that the vaso-constrictor center was not active (we are not speaking of degree of activity now).



Fig. 3.—Same rabbit as shown in Fig. 2. Photograph taken later in the experiment after the vessels of the left ear had also been cut off from central control. The formerly well contracted vessels of the left ear are now well dilated. (See Protocol V.)

We have three experiments which present a variation from the above type and which throw an interesting light upon this struggle of the vaso-motor center for control of a desperate situation. We may describe this type as one in which the slope, representing the general condition of the animal, is interrupted at one point by a sudden sharp descent of the line, followed by a more or less rapid return of the line to the level of the original slope. Such an experiment is the following:

PROTOCOL V.

Experiment 28. July 15, 1914. Male White Rabbit. 1550 gm. Shock experiment started 24 hours after denervating vessels of right ear.

8:05 A. M. Vessels of denervated ear well dilated. Those of normal ear well constricted.

8:10	Operation began. Method exactly same as in Experiment 34 (Protocol III).
8:50	Operation finished. Lid reflex good. Respiration regular and efficient.
9:03	Blood pressure 34 mm. Hg. Vessels of denervated ear well dilated. Normal vessels well constricted.
9:15	Considerable blood lost accidentally from axillary artery. Struggling. Heart and respiration stopped. Lid reflex gone. Clamped aorta above celiac. Started artificial respiration and cardiac massage.
9:19	Recovering well now.
9:25	Just before animal nearly died, at 9:15, the normal ear was practically blanched, while the denervated vessels were well dilated. Then at 9:19, with the recovery of the animal, the vessels of the normal ear became widely dilated (no vaso-constrictor tonus evident at all). Now the normal vessels are already beginning to constrict. They are definitely smaller than the denervated vessels again.
9:32	Vessels of denervated ear well dilated. Normal vessels practically blanched. Aorta still clamped. Blood pressure 92 mm. Hg.
9:40	Took photographs of ears as at 9:32 (Fig. 2). While taking the photograph, observed that the vessels of normal ear showed excellent rhythmic contractions and dilatations at the rate of 6 per minute. The vessels oscillated between "blanched" and "slightly constricted." The rhythmic changes easily seen at a distance of three feet. Denervated vessels never showed rhythmic contractions.
10:10	Must leave clamp on aorta, otherwise blood pressure quickly falls to near zero and heart stops. Cut left cervical sympathetic and removed ganglion.
10:20	Vessels of left (normal) ear already wide. More photographs (Fig. 3).
10:32	Blood pressure 96 mm. Hg. with aorta clamped.
10:37	Clamp off aorta.
10:38	Blood pressure 12 mm. Hg. Heart stops if clamp is removed from aorta for any length of time. Lid reflex nearly gone.
10:38½	Heart nearly stopped. Clamp on aorta again.
10:45	Lid reflex good. Respiration very slow, regular, and apparently efficient. Blood pressure has risen to 35 mm. Hg. Vessels of left ("normal") ear (denervated at 10:10) slightly wider even than "denervated" vessels. Heart stops quickly whenever clamp is removed from aorta. Experiment discontinued.

Here we see an animal in which (until 9:15) the course of the descent into a state of shock was in no essential particular different from the usual one, which has already been described. At 9:15 an accidental hemorrhage nearly resulted in the death of the animal. Both the heart and respiration stopped and the lid reflex disappeared. Within four minutes, however, through the use of artificial respiration and cardiac massage, the animal had revived and was doing fairly well. The blood vessels of the normal ear, which before the hemorrhage were strongly constricted, showed now a marked contrast to that condition. They were widely dilated, which means that there was little if any evidence of vaso-constrictor tonus. From this time on, however, the vessels gradually became smaller and smaller. At the end of six minutes they were already definitely smaller than those of the control (denervated) ear. At the end of thirteen minutes they were again as strongly constricted as before the hemorrhage. One of the photographs taken shortly after this (Fig. 2) shows something of the contrast between the vessels of the two ears, though it is by no means as striking as the actual picture presented by the ears themselves. The reason for this is twofold. First, the smaller vessels of the denervated ear that were visible to the naked eye do not show up distinctly and the opacity of the larger vessels along their margins seems to have been insufficient to cast a distinct shadow upon the photographic plate. As a result these (larger) ves-

sels seem narrower in the photograph than they actually appeared to the eye. Second, the photograph fails entirely to show the color differences of the two ears. The engorgement of the denervated ear was not confined to the larger vessels, but seemed to involve also those that were invisible to the eye for the whole ground substance of this ear had a definite and marked pinkish flush. The normal ear, in contrast to this, had a cadaveric whiteness which was tinged with a suggestion of yellow rather than of pink. In addition to this blanching of the ear substance, the smaller vessels were invisible and the larger ones reduced to mere threads.

In order to convey a more accurate impression of this contrast, we had the artist make a water color sketch from life (see Fig. 1). While it is of necessity partially schematic so far as the vessels of the ears are concerned, still it gives a much more exact general impression than does the photograph.

Both the photograph (Fig. 2) and the colored sketch (Fig. 1) represent the appearance of the ears at a time when the general blood pressure was so low that the heart stopped when the clamp was removed from the aorta, thus allowing the splanchnic area and the posterior part of the body to be included in the circulation. With the aorta clamped just below the diaphragm, however, the heart built up a pressure in the head region equal to 92 mm. of mercury, and in the face of this pressure the vessels of the normal ear remained constricted as shown in the two figures just mentioned.

A little later in the experiment the vessels of the normal ear were also denervated, whereupon the constriction of these vessels was quickly replaced by a wide dilatation. Fig. 3 is one of the photographs taken at this stage of the experiment. While it fails to bring out the flushing of the ear substance that developed, it does show fairly well the enlarged blood vessels.

The description of the contrast between the normal and the denervated vessels just given holds in a general way for all the experiments in the series. The individual variations that were seen were variations in the degree of the contrast. When "one day" rabbits were used, and the aorta clamped temporarily late in the experiment, the above described differences obtained in by far the majority of the experiments.

As already stated, Protocol V is one of a group of three experiments in which there was evidence of early vaso-constrictor failure. Of the two remaining experiments, which belong to this group, one was in all respects similar to Protocol V. That is, there was only a temporary loss of vaso-constrictor activity after stoppage of the heart, and this was followed by a good recovery. In the third experiment, although the animal recovered sufficiently to breathe without artificial respiration, it was not possible at any time thereafter to remove the clamp from the aorta without causing stoppage of the heart. This was apparently due to the fact that the venous return to the heart was insufficient, for upon removing the aortic clamp the arterial pressure quickly fell to a very low level, e. g., from 62 to 14 mm. Hg., and soon thereafter the heart would slow up and stop. Moreover, during the remainder of the experiment the vessels of the normally innervated ear reacted to internal pressure changes in exactly the same way as those of the denervated (control) ear. All of which means that after the temporary stoppage of the heart and respiration early in the experiment the vaso-constrictor center never gave any evidence of recovery.

We have made no systematic study of the effects of hemorrhage or temporary cardiac failure upon the resistance of the vaso-constrictor center. But the results obtained in these three experiments indicate that if such accidents complicate the situation the behavior of the center is markedly different from what it is in those cases in which shock is produced by operative trauma alone.

We appreciate well the fact that in such studies as this it may always be disputed that the condition studied was really that of surgical shock. We wish, however, to call attention to this important point in connection with our experiments. *It is not essential that our animals be reduced to a condition which will be acknowledged by everyone to be identical with deep surgical shock as usually encountered.* We were convinced that our animals sank finally into such a state. It must be recalled, however, that our problem was to determine whether during the *course of development of shock* the loss of vaso-constrictor tonus is the primary cause of all the remaining symptoms, or whether this tonus persists until other symptoms have become marked. Whether in fact, the activity of the vaso-constrictor center may not be well up to normal at a time when the general blood pressure has fallen to a low level and the other symptoms become strongly developed. This being the case, it was only essential that our animals be well on the way toward a condition of deep shock, and it can hardly be disputed that such was the case.

The method used in these experiments to demonstrate the presence or absence of tonus in the vaso-constrictor center could, we believe, be used as a means of demonstrating vaso-constrictor activity in other lines of experimentation just as well as in shock.

Furthermore, since we have (within certain limits) means for raising the blood pressure at will, the *degree* of activity of this center could be determined by raising the blood pressure until the normally innervated vessels begin to yield, i. e., dilate, under the pressure. We might term this the "breaking point." It might even be possible, by making a series of such determinations during the course of a single experiment, to plot a curve that would represent the behavior, as regards degree of activity, of the vaso-constrictor center.

It has been stated⁶ that the left ear of the rabbit is better supplied with vaso-motor nerves than is the right. If this be the case, it would be better in experiments such as these to denervate the already poorly innervated right ear and use it as the control. In nearly all our experiments the right ear was used as the control.

SUMMARY AND CONCLUSIONS.

1. Denervated blood vessels usually show the best passive response to internal pressure changes when used within twenty-four hours after their denervation.

2. Normally innervated ear vessels are strongly constricted while the animal is sinking into a condition of shock and after shock has developed.

3. This strong vascular constriction persists even though the blood pressure be raised well toward the normal level (at a time when the animal shows distinct symptoms of shock).

⁶Meltzer and Meltzer: Am. Jour. Physiol., 1903, ix, 66.

4. This vaso-constriction is due to the activity of the vaso-constrictor center because (a) the control denervated vessels become strongly dilated under the influence of the same pressure and (b) the constriction of the normal vessels themselves disappears at once if their connection with the vaso-constrictor center is destroyed by cutting the nerves, or by abolishing their conductivity with ether or by freezing.

5. Since a fairly high degree of activity of the vaso-constrictor center can be demonstrated even after the blood pressure has fallen to a low level and reflexes are sluggish we believe it is justifiable to conclude that a paralysis or failure of the vaso-motor center is not the *primary* cause of the other symptoms of surgical shock.

THE PHYSIOLOGY OF THE PARATHYROID GLANDS*

By W. F. KOCH, DETROIT, MICH.

THERE are in the animal organism a number of epithelial bodies occupying definite anatomical positions. Some of these structures which we call glands, possess duct systems which direct the product of metabolism of their epithelia into some body cavity where this product or secretion carries on a definite physiological activity. On the other hand a number of these epithelial bodies are without ducts so that no products of their metabolism can be directly collected. These are the ductless glands and because of the intimate relation of their cells to the lymph and blood capillaries they have been supposed to elaborate substances which are carried by the blood or lymph streams to the various cells of the body. They have been named therefore the glands of internal secretion. The question of their physiological significance has interested the biologist for many years. Yet up to the present day very little light has been thrown upon the mechanism of their activities.

A brief reference to their phylogenesis will show that they are specializations of protoplasm, developed so as to serve better the requirements of the cells of the complex multicellular organism. Naturally with a development of the organism, from the simple single cell to the large structure of many millions of cells, which may be widely separated and therefore placed in different environments, the variously located cells must not only carry on the vital processes of their simple progeniture, but also according to the principle of adaptation they must specialize in those activities which their environment requires for their best service to the organism. Thus the surface cells become protective; certain other cells become highly contractile and accomplish locomotion. Now with this specialization in function of any cells the generality of function as found in the unicellular organism must be to some extent sacrificed. And the specialized cell becomes dependent, perhaps parasitic, upon other cells for a portion of the activities which it no longer finds opportunity to carry out itself; thus the principle of mutual service is developed. And it may be assumed that

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the glands of internal secretion because of their relation to the blood stream, through this medium, supply vitally important substances to the other cells of the body. We find a concrete example in the chemical structure of the thyroid apparatus. In sponges which have no thyroid gland considerable iodine is found in the skeletal structures. In the higher organism, fishes, mammalian, etc., there is a thyroid gland containing considerable iodine and the skeletal structures contain it no longer. It may be assumed, then, that the thyroid gland with its iodine compound does that metabolic work for the skeletal structures which they once performed themselves when they contained this iodine body. Confirmation of this assumption is observed in diseases of the thyroid apparatus where abnormalities in the skeletal tissues result. The physiological relation of adrenalin, found in adrenal glands, to the sympathetic nervous system substantiate further the interdependence of the various tissues and the service the glands of internal secretion may pay those other tissues which work more directly to the adaptation of the organism. Using the above assumption as a guide the writer has endeavored in this work to elucidate the mechanism of activity of the parathyroid glands which up to the present research though given much attention has remained obscure.

WORKING BASIS AND HISTORICAL SKETCH.

The thyroid glands are two oval bodies located ventrally and laterally to the thyroid cartilage and trachea in the neck. In the neighborhood of these larger glands, or imbedded in them, are four small epithelial bodies. These are the parathyroids. They may be grouped in two pairs, the superior pair being located about the superior poles of the thyroid; the inferior pair generally at the inferior poles. They are flattened, round or oval in shape, and in the dog together present a mass no larger than a lentil. Because of their small size and irregular occurrence, they were not discovered until 1880, when Sandstrom called attention to their individuality.

Histologically, their structure differs greatly from that of the thyroid, with which they are so closely associated. They receive their blood supply from branches of the thyroid artery. Their veins enter into those of the thyroid. The glands have a capsule of connective tissue, which, supporting the larger vessels, dips into the glandular substance, imperfectly separating it into lobules. These lobules may take the form of columns of cells, which anastomose; and these columns may be made of one or several rows of cells, sometimes grouped into round follicles. There are three varieties of cells uniformly observed. Two of these types are larger than the thyroid cells having deeply staining nuclei and slightly staining protoplasm with fine boundaries quite distinct. The other type resembles the thyroid cell being low columnar and arranged on a basement membrane which surrounds, follicle-like, a small lumen, that may be filled with a granular or colloidal material. Of the former type, a minority of the cells are large polygonal with deeply-staining nucleus and deeply-staining granular protoplasm, which because of its acidophil nature, resembles some of the hypophysis cells. These several varieties of cells may perhaps represent, by their differences in appearance, various stages in functional activity. Very striking is the richness of the blood supply. This is of the sinusoidal type. The thin endothelium of the blood capillaries is placed in close apposition to practically the whole

surface of each cell from which it is not even separated by fibrous tissue strands. Frequently, glands are found in which the epithelial cords are split by the accumulation of colloidal material and thus an acinus is developed, which resembles closely the follicles that make up the thyroid gland. For this reason the parathyroids have been looked upon for some time as embryonic thyroids.

Whether they have a physiological significance differing from that of the thyroids was disputed for some time, since their supposedly complete removal from the animal body was not always followed by definite physiological changes. This is not surprising for a complete surgical removal of the glands is sometimes impossible. Occasionally one of the glands may be found in relation to the thymus, or in some other part of the mediastinum. We have learned, moreover, through the work of Halsted,¹ that only a minute portion of parathyroid tissue is capable of rapidly growing sufficiently large to carry on the work of all the glands. This was proved by carefully removing all the glands that could be found in a favorable experiment and then transplanting a small piece of a parathyroid in the abdominal wall. It was found that with this small piece of grafted parathyroid tissue the animal behaved quite normally for a long time. After the transplant was removed, however, the typical complex of symptoms developed, which characterizes the parathyroid insufficiency. It is not surprising, therefore, that the older observers (Forsyth) looked upon the parathyroids as incompletely developed accessory thyroids, supporting this view with the observation that after removal of the thyroids, the parathyroids rapidly changed their structure to resemble that of the thyroids. In order to explain the frequent appearance of the syndrome of parathyroid insufficiency, they assumed that such changes were brought about by injury to the superior laryngeal nerve, or other tissues of the neck region (Munk).

The capacity of the parathyroid to take up the thyroid function does not exclude, however, an independent significance; and that these glands mean something more than the thyroids was shown by Beidl, Moussu,² Glay,³ Vassale and Generale,⁴ when they pointed out that the removal of the thyroid produces simply a condition of cachexia and the changes associated with myxedema, whereas, removal of the parathyroids is responsible for a typical nervous symptom-complex.

This syndrome, though often referred to as typical, has been rather incompletely described in the literature. Several detailed protocols are, therefore, submitted. The behavior of the parathyroidectomized dog may coincide with either of two distinct types of symptoms, or with a mixture of these types, in which either may predominate. In one type the dominant feature is over-excitability; in the other under-excitability. In the former tonic convulsions are characteristic; in the latter we observe a peculiar muscular flaccidity and a general depression of the nervous system. In either case a pathological condition develops within one or a few days after removal of the glands and proves fatal within two to ten days. The first type is illustrated in the following protocol:

Dog (3) was completely parathyroidectomized December 6, 1913, at 7 p. m. At 8 a. m. December 7th, he had recovered from all visible effects of the anesthetic and operation. He seemed fairly bright and active until 11 a. m. December 8th. At this time the first symptoms of tetany were noticeable in a wrinkling of the forehead and twitching of the right ear. At 12 o'clock the

twitchings were visible in the shoulder muscles and the hind limbs were somewhat extended. During this time the heart rate had increased to 160 per minute; the respiration became more rapid and somewhat labored. At 2 p. m. the dog was found in complete tetany, lying on his side with limbs extended and with opisthotonos; the jaws were locked and the lips raised. The eyes, because of retraction of the lids, seemed to bulge out, the pupil was dilated and the sclera injected. Salivation and lacrimation were profuse. During this convulsion the respirations were extremely difficult, each inspiration and expiration producing a sound suggestive of laryngeal spasm. Inspirations were deep and expirations seemed incomplete and difficult. At about 2:15 the dog attempted to rise to his feet, but his struggles were futile since flexion of the limbs was impossible. By 2:50 the respirations had become shorter and more rapid with the production of less sound, and it was noticed that although the hind legs were extended, one of the fore limbs was now flexed, and instead of being in general tonic spasms, the muscles now played in twitches. A similar twitching was also observed in shoulder and trunk muscles. By 3:30 the respiration changed to a rapid panting; the muscles of the fore limbs were more flaccid, while those of the hind limbs exhibited twitches. The face still retained a peculiar expression with raised lips, wrinkled nose and brow and ears drawn back. He rested in this condition until about 7 p. m. when another and similar convulsion developed which did not subside until after midnight. At 6:30 a. m. the subject was found dead in the cage in an attitude of opisthotonos. Necropsy showed a hyperemia of all of the viscera. The ordinarily invisible intestinal vessels were so dilated as to be easily traceable, the liver and spleen were markedly congested, the bladder filled with urine and the intestinal tract with fluid.

The most striking histological changes occurred in the blood, liver, kidney and brain. The blood of the vena cava and heart of this and all animals showed extensive ante mortem coagulation. White clots in several cases were continuous from within the heart chambers down the vena cava to its iliac bifurcation. They nearly filled the lumen of the vessel. Upon section of the liver, the vessels showed fragmented erythrocytes, many normoblasts, erythroblasts with mitotic nuclei and a small proportion of erythrocytes that stained brilliantly in eosin; the remaining red cells in large areas were blood shadows. Each section of the liver and lung showed a number of large mononuclear cells with eosinophil granules. There were also present a larger number of large flat cells staining very intensely in eosin. These showed no definite granulation. In places they were found to line the smaller veins like endothelial cells. In these places no endothelial cells could be observed. The cells of the hepatic cords showed advanced fatty degeneration of the protoplasm. The nuclei of large areas had disappeared entirely in places where the cell form was fairly well preserved. Such areas were surrounded by circular areas of cells in which the nuclei had become densely stained clumps of chromatin. In the livers of four of the dogs only a diffuse chromatolysis could be observed.

All kidneys showed marked congestion and hemorrhage in the cortex, some anemic, and others, congested medullæ. Some glomerula had lost Bowman's capsule and were hemorrhagic, others were markedly congested. In some of the convoluted tubes the epithelium had degenerated.

The spleen contained a large quantity of pigment. Some of the cells showed chromatolysis.

The lung showed edema, congestion and the blood changes mentioned.

The brain sections, which I prepared in Professor Barrett's laboratory, showed cells in the motor areas with partial loss of Nissl substance and typical tetany nuclei. Various degrees of chromatolysis were also observed in these nuclei.

The intestinal tract besides marked congestion showed in the duodenum and pyloric end of the stomach disintegrating epithelial cells. Their nuclei were converted into solid deeply-staining clumps. These appeared like those in the process of extrusion from the normoblasts.

It is observable that the totality of the symptoms points to a hyper-excitability of the whole nervous system including those neurons contributed by the cord to the sympathetic system. Now the action that may elicit so striking a positive phase, may also be expected to present a negative phase in which a general depression of the central nervous system results. Out of 47 dogs, 2 such cases were found. The following protocol will illustrate:

Dog (31) was operated upon March 8, 1914; at 2 P. M. it recovered from the anesthetic and was apparently normal until March 10th, when instead of walking about the cage and welcoming its attendant, it exhibited no recognition of his presence. It was examined and found lying asleep in a peculiar state of flaccidity with limbs somewhat flexed. When a limb was moved or the head turned back it retained the attitude given it. The subject was not observed to move during the day. It remained in this condition until March 12th when, at 2 P. M. it was found dead in the attitude of dogs that die in tetany. A post-mortem examination revealed no signs of pneumonia or other infection. We, therefore, believe that the dog succumbed to parathyroid insufficiency. This case presents perhaps an over-stimulation of the central nervous system, comparable to shock, a state which may be compared perhaps to the reversing of a chemical reaction by the products of the reaction.

The regular occurrence, after complete parathyroidectomy, of a typical symptom-complex facilitates the study of the mechanism by which these glands functionate, since a study of the causation of the tetany should reveal the position of these glands in the metabolism of the organism. Previous to this research, only one contributing fact has been brought forth; it is the discovery by MacCallum⁵ that the urines of parathyroidectomized animals contain excessive quantities of calcium; and that when calcium salts are injected intravenously into such animals, the tetany is immediately controlled. MacCallum⁶ expressed the view therefore, that the parathyroids had to do with the metabolism of salts but more especially the calcium salts; and he referred to this substance a special physiological value, such that when it was lost from the animal body a calcium deficiency resulted that constitutes the essential pathological condition of parathyroid insufficiency. It was shown by Beebe⁷ and Beebe and Berkeley⁸ that injections of other salts have similar though not so marked an effect. These observers showed that the length of time over which aqueous solutions of calcium or other salts are useful in controlling the tetany, is relatively short, varying between one and several days. It appears that if calcium insufficiency were the essential change the addition of calcium to the body through intra-

venous injection should alleviate the pathological condition so long as this treatment was used.

In a recent investigation the writer found that when the tetany became uncontrollable through injections of aqueous salt solutions the kidneys had become so pathological as to be unable to functionate normally. Since one of the effects of such intravenous injections is diuretic, aiding the elimination of toxic substances from the blood, it may be assumed that one of the beneficial effects of the aqueous calcium injections depends upon increasing the work of the kidneys and thus the detoxication of the blood. That calcium should herein be more valuable than other salts may depend upon its depressing qualities, but the fact that it is a diacid base would indicate a value as an acid carrier greater than that possessed by the monovalent metals. Therefore when excessively excreted from the body a large number of acid radicals are lost and when injected a large content of acid radicals is added to the blood. If then the value of calcium depends upon the increasing or maintaining of a certain reaction of the blood, the acid radicals are here the important factors. They present two possible modes of activity, the simple neutralization of basic substances excessively elaborated, within the body or the destruction of such substances as are capable of producing the tetany.

There is still another source of indications that aid in the directing of the present investigation. We expect that when a vital process is removed from the organism, the dependent processes will come to a standstill. If this be true some hitherto useful substance should be present and excreted from the organism unused as fast as it is offered for metabolism. Such a substance must contain vitally reactive groups, and if these groups are not normally taken care of they present the possibility of disturbing other vitally reactive substances in the organism and thus of becoming toxic.

There are then several indications that the tetany of parathyroid insuffi-

RECORD A-I. (See opposite page.)

(Dog 1)

Injections given by jugular vein.

Quantity—5 c.c. 0.6 mgms. of natural substance.

Fall in pressure from first injection.....about 30 mm. Hg.

Fall in pressure from second injection.....about 5 mm. Hg.

Fall in pressure from third injection.....about 20 mm. Hg.

Heart rate before injecting.....114 per minute.

Heart rate after injecting.....114 per minute.

Interval between injection 1 and injection 2.....about 72 seconds.

Interval between recovery from first and administration of second injection, about 36 seconds.

Interval between injection 2 and injection 3.....about 45 seconds.

RECORD A-II. (See opposite page.)

(Dog 1)

Injections given by jugular vein.

Quantity—5 c.c. 0.6 mgms. of synthetic substance.

Fall in pressure from first injection.....about 25 mm. Hg.

Fall in pressure from second injection.....0 mm. Hg.

Heart rate before injecting.....138 per minute.

Heart rate after injecting.....136 per minute.

Interval between injection 1 and injection 2.....about 60 seconds.

Interval between recovery of first and administration of second injection..about 30 seconds.

Record I Dog I natural subst.

my. 1.

my. 2.

my. 3.

RECORD A-I.

agathae subst. → trigular

my. 1.

my. 2.

RECORD A-II.

ciency is due to an intoxication. Namely, that it is subdued by increased diuresis and by the neutralization of toxic basicity or the destruction of a toxin by acidity. That the origin of the hypothetical toxic substance is the body itself, that it is useful and not toxic in the presence of the parathyroid glands, that it is filterable through the glomerulus of the kidney (and thus readily diffusible as is shown by the value of diuresis in controlling the tetany) point to a substance hormone-like in nature and therefore very unstable chemically.

It is the object of the present investigation to ascertain the presence and identity of such a substance in the urines of parathyroidectomized dogs and study its physiological properties. As may be anticipated the isolation of an unstable substance from the complex urine presents several difficulties which exclude the hope of quantitative results. My object therefore was to isolate this substance in only sufficient quantities for identification and experimental purposes. After a careful study of the urines the following method was adopted. Its special advantages depend upon its simplicity, rapidity and the avoidance of destructive chemical reagents.

THE METHOD OF ISOLATION.

The urines were collected separately from forty-seven parathyroidectomized dogs. Especially designed cages were used to avoid fecal contamination. The urines were filtered and evaporated to a syrup by an electric fan at a temperature not above 20° C. The residues were dissolved in alcohol, filtered and evaporated, and this process repeated until the last evaporate dissolved readily in alcohol. The lipoids present were extracted with ether and the residue taken up in water. This solution was cautiously precipitated with picrolonic acid. Several insoluble picrolonates were thus obtained, and by recrystallization from water and alcohol were purified.

These substances were tested for physiological activity. Two of them were found to modify the blood pressure when injected intravenously into anesthetized dogs. When injected intraperitoneally into non-anesthetized animals they exhibit very marked toxic effects. They were therefore selected for analysis.

One substance was found to reduce gold chloride quite rapidly, and picrolonic acid slowly. I therefore tested for an aldehyde group. With ammoniacal silver nitrate no mirror was obtained but instead a yellow gray precipitate whose solubility resembles that of silver cyanide. The substance itself freed from picrolonic acid is practically neutral in reaction, very soluble in water and alcohol, somewhat soluble in ether. It gives a picrolonate in the form of very fine

RECORD B. (See opposite page.)

(Dog 2)

Injection given by femoral vein.

Quantity—5 c.c. 0.4 mgms. of synthetic substance per kilo body weight.

Fall in pressure from first injection.....about 25 mm. Hg.

Fall in pressure from second injection.....about 10 mm. Hg.

Interval between injection 1 and injection 2.....about 50 seconds.

Interval between recovery from first injection and administration of second, about 20 seconds.

Note: The heart rate cannot be estimated because while taking the tracing, the recording pointer of the chronograph fell off. After the tracing was finished, we attempted to supply a time record, but it did not prove to be synchronous with the former rate of movement of the drum, which was running down. The time record is therefore valueless.

Log-II Symbolic Subst.

ing 1

ing 2.

RECORD B.

microscopic needles which melt at a 118° C., solidifying rapidly to an orange-colored mass which melts with decomposition at about 230° C. 0.249 gms. are soluble in 100 c.c. of hot water.

Upon analysis it gives the following percentage composition.

0.1060 gms. substance gives 26 c.c. N. at 24° C. and 746 mm.
0.1783 gms. give 0.2933 gms. CO₂ and 0.0696 gms. H₂O.

FOUND	CALCULATED FOR	
	(C ₂ H ₄ N ₂)	(C ₁₀ H ₈ N ₄ O ₅)
N 26.49	26.31	
C 44.86	44.97	
H 4.34	3.75	

The substance agrees in percentage composition with the picrolonate of methylcyanamide. Methylcyanamide was prepared synthetically from methyl mustard oil. It resembles the naturally occurring substance both in reaction and solubilities. Its picrolonate was prepared and found to melt at 116° C. solidifying immediately to an orange-colored mass which melted at 230° C. with decomposition. In this respect the picrolonate agrees in its behavior with the picrolonate of the naturally occurring substance.

The other substance isolated from the urines and having a physiological action is basic in reaction. It is less soluble in water than the first substance. Its picrolonate crystallizes from water forming small orange-colored mounds. The picrolonate melts at 232° C. with decomposition. It gives on analysis the following percentage composition.

0.1096 gms. give 26.2 c.c. N. at 24° C. and 750.7 mm.
0.1288 gms. give 30.2 c.c. N. at 22.5° C. and 751.3 mm.
0.1731 gms. give 0.2827 gms. CO₂ and 0.063 gms. H₂O.
0.1345 gms. give 0.0520 gms. H₂O and 0.2184 gms. CO₂.

FOUND		CALCULATED FOR	
A	B	(C ₆ H ₁₂ N ₆) ₃	(C ₁₀ H ₈ N ₄ O ₅) ₃
N 26.6	26.28	26.31	
C 44.54	44.28	44.97	
H 4.07	4.32	3.75	

The substance agrees in composition with the picrolonate of trimethylmelamine which is the polymer of methylcyanamide. The polymer was prepared from the synthetic methylcyanamide and found to agree in reaction with

RECORD C. (See opposite page.)

(Dog 2)

Injection given by femoral vein.
Quantity—5 c.c. 0.4 mgms. of synthetic substance per kilo body weight.
Fall in pressure from injection 3.....about 25 mm. Hg.
Fall in pressure from injection 4.....about 0 mm. Hg.
Fall in pressure from injection 5.....about 0 mm. Hg.
Heart rate before injecting.....108 per minute.
Heart rate after injecting.....108 per minute.

ing. 3.

ing. 4

ing. 5.

Record III Aug. II Synthetic subet.

RECORD C.

the natural substance. The picrolonate of the synthetic polymer was found to melt at 229.4° C. which is slightly below the melting point of the natural salt. This discrepancy can be explained by the presence of small quantities of methylguanidin in the synthetic preparation which owing to the similarity in solubility of the two picrolonates, could not be completely removed by fractional recrystallization. It was found that methylcyanamide polymerizes after a few days' standing or several evaporations of its watery solution. Therefore the polymer should be expected in the urine from which the cyanamide was isolated.

Because of the agreement in chemical and physiological properties (discussed below) the substances may be considered identified as methylcyanamide and trimethylmelamine.

PHYSIOLOGICAL PROPERTIES.

The methylcyanamide isolated from the urines and the synthetic methylcyanamide were injected intraperitoneally in non-anesthetized dogs and found to have similar effects. In small doses they produce extreme vasodilatation observed in the reddening of the sclera and swelling and reddening of the tongue. Larger doses cause paralysis and convulsions. Still larger doses cause an extremely rapid death. Intraperitoneal injection of 27 mgs. of the synthetic substance into a rabbit of one and one-half kilos' body weight produced after sixteen minutes very marked vasodilatation observable in the air vessels, and a very labored breathing accompanied by wheezing suggestive of bronchial stricture. In this condition the animal rapidly developed tremors and tetany with the head thrown back and hind limbs extended. After a few minutes the tetany gave way to coma resulting in death. An injection of 22 mgs. of natural substance in a rabbit of one and one-quarter kilos produced death very similarly although the tetany was not marked and the dyspnea and coma symptoms predominated. On the other hand 4 mgs. injected intraperitoneally into a white rat of about a 100 gm. weight caused death practically instantaneously with opisthotonos developing no coma whatever.

In order to obtain the base for injection the picrolonate was dissolved in a small quantity of alcohol and decomposed with a calculated quantity of sodium carbonate. The picrolonic acid is thus precipitated as a sodium salt and filtered off. The filtrate was evaporated by an electric fan to dryness and the residue dissolved in water. In this way a solution of known concentration was obtained. The synthetic substance was prepared fresh each time it was used, and from a known quantity of methylthiourea.

Blood pressures were taken by canulæ in the carotid, from dogs anesthetized with chlorotone. For record A-I, the natural substance, for record A-II, the synthetic substance was injected into the jugular vein. For records B and C, the synthetic substance was injected into the femoral vein, experience having shown that injecting into the jugular disturbed the canula.

Record A-I shows the effect of injection of an aqueous solution containing 0.6 mgms. of substance per kilo body weight. Five cubic centimeters of this solution were given with each injection. The first injection was immediately followed by a sharp drop of about 30 mm. of mercury in blood pressure. A second injection of the same quantity given fifteen seconds after recovery from

the first showed only a slight change; a third injection following the second, by the same interval, had an effect less than the first and more than the second. It appears that the animal had become noticeably refractory to the substance after the first injection, and had recovered from this refractoriness considerably at the time of the third. This refractoriness is observed whether the natural or synthetic substance is injected, and its duration toward either substance varies with the animal. In our experience older dogs have a longer refractory period than younger dogs. The dog from which record A was taken recovered almost completely after 140 seconds; the dog from which record B was taken required between 80 and 360 seconds for a complete recovery.

To explain this refractoriness and the quantitative relations discussed below, it might be assumed that the methylcyanamide acts in conjunction with some receptor substance slowly elaborated by the body to produce the vasodilatation and thus the fall in blood pressure. The refractoriness would then be due to exhaustion of the receptor.

In record B the first injection did not use up all of the receptor since an injection following by about 25 seconds, the return of blood pressure to normal, still produced an effect. That detectable receptor is not generated in this dog in 25 seconds is shown in record C where injections 4 and 5 caused practically no pressure change though given at much longer intervals. It appears therefore that injection 4 produced its fall by working with excess of receptor not used by injection 3. The above described relations of the cyanamide and the receptor indicates that they react quantitatively to produce the fall in blood pressure.

It is observable in all of the tracings that any excess of the cyanamide not reacting with the receptor to produce a fall in blood pressure is nevertheless rapidly disposed of, since where sufficient time has elapsed for the generation of noticeable quantities of the receptor, no fall in pressure occurs until a further injection of the cyanamide is given. It may be this reaction of the cyanamide in combining with other groups in the organism that produces the tetany and other symptoms leading to the death of the animal.

It seems that the substance is excreted by the parathyroidectomized animal in considerable quantity. For despite its instability, from the urines of 47 experimental dogs 1.2 gms. of the cyanamide were isolated and 2 gms. of the polymer, as picrolonates. The quantities actually present in the urines or the portions decomposed during the isolation cannot be surmised. Nor would such data be an index to the quantity generated in the organism since during the process of intoxication a considerable portion would be disposed of.

A comparison of the toxicities of the urines of a dog before and after parathyroidectomy shows that during non-fatal tetany the urine is somewhat toxic but that after fatal tetany, the urine is much more toxic. This is shown by the following experiment: A dog weighing 18 kilos. was parathyroidectomized. Eight c.c. of the urine excreted before operation, produced no marked toxic symptoms when injected into a rat of about 100 gms. Five c.c. of 90 c.c. of urine excreted during the half day in which the dog was in moderately severe tetany, upon injection into another rat of the same size produced mild opisthotonos from which the rat recovered in about ten minutes. The urine, after death, in a quantity of 5 c.c. produced severe and almost fatal tetany upon in-

jection into a third rat of equal weight. The urines obtained between tetany periods in quantities of 5 c.c. were not toxic. It may be approximated then that a dog of 18 kilos, and excreting about 200 c.c. of toxic urine gives off from 10 to 80 mgms. of the cyanamide, if we assume that the 5 c.c. injected contain about 1 to 2 mgms. of the substance since 4 mgms. is toxic to a rat of equal size.

The similarity in the behavior of the parathyroidectomized dogs, to that of the non-anesthetized animals treated with the substance isolated from the urine, is further indication that this substance is responsible for the symptom-complex of parathyroid insufficiency. The data therefore justifies the following conclusions:

1. Somewhere in the body methylcyanamide is generated.
2. This substance has a physiological value in normal animals.
3. After parathyroid extirpation the substance accumulates to toxic quantities, and is responsible for the death of these animals.

A further study of some of the problems developed from this investigation is receiving attention.

OPERATIVE AND POST-OPERATIVE PROCEDURES.

The operation consists of complete removal of the thyroid and parathyroid glands, together with the surrounding capsular tissue. The essentials of the operative technic are:

1. Thorough asepsis.
2. Prevention of post-operative hemorrhage.
3. The least possible shock and impairment of vitality.

The aseptic methods are those common to most operating rooms and scarcely require detailed description. The same rigid precautions must be observed as in any major operation. The reason for this care is that the oozing of blood and lymph from the rich capillary bed in which the glands are implanted produces ideal media for the development of pyogenic organisms. This is to be avoided not only because the well-being of the animal is reduced by such infection, but also because it is desirable to obviate as far as possible any discharge from the wound. Another feature not to be ignored in the matter of bacterial growth in the wound is that the absorption of its products may affect the constituents of the urine. A specially devised mask that covered the muzzle of the animal but could not slip down on the field of operation was used. The field of operation was shielded from the mask and hands of the anesthetist by a sterile cover. All the loose dirt and hair are removed from the animal's body by a thorough scrubbing with soap and water and a subsequent drenching with warm 1-1000 bichloride of mercury solution. This is done some time prior to the operation.

To prepare the field of operation, the animal's neck is shaved (over the entire anterior aspect) from the angle of the mandible to the juncture of the neck with the thorax. This is best done immediately prior to the operation. After shaving, the skin is scrubbed with soap and water, dried with alcohol, and then painted with iodine.

Operation.—The following landmarks must be identified: hyoid bone, sterno-

mastoid muscles, larynx, thyroid notch, and trachea. The operator must carefully keep the animal's head directly in line with the rest of the body or the relationship of the parts will be distorted.

Incision is made from a point exactly at the middle of the inferior border of the hyoid bone to a point in the midline at the third ring of the trachea. The first point may be determined by palpating the notch, between the two thyroid cartilages of the larynx, which locates the midline precisely. Such an incision will be about three inches long. The superficial cervical fascia is then incised by gently drawing the knife over its surface. This exposes the platysma fascia which is cut in line with the original incision. It is better to cut through these fascia separately as I have described, because occasionally the anterior jugular vein or some of its larger branches cross the line of incision, and these may be clamped before cutting through the platysma fascia. Thus no hemorrhage occurs to obscure the field or cause unnecessary delay. Passing through the platysma fascia or superficial layer of the deep cervical fascia, the infrahyoid muscles overlapped by the sternomastoid muscles lie in close proximity on either side of the midline. The point of a four-inch scissors is then inserted in the furrow between the inner borders of the two muscles and the intermuscular septum severed by spreading the scissors. This septum is more readily identified if care has been taken to make the previous incisions in the midline and the animal's neck has been retained in the anatomical position. The last step brings us down to the surface of the trachea and larynx covered only by the middle layer of the deep cervical fascia. In the dog, the thyroid gland is represented by two lobes, one on either side of the trachea, each lying in a fibrous sheath derived from the middle layer of the deep cervical fascia. As a rule there is no isthmus or pyramidal process as in the human thyroid. There was a marked variation in size of the individual thyroids we examined as well as in their position, some being immediately below the larynx and others occurring even an inch lower.

One of the lobes is now sought for and brought up into the wound together with the loose fibrous tissue attached to it. It is then seized with a strong hemostatic forceps and light tension is brought to bear on the two poles by which it is fastened. The main branch of the inferior thyroid artery, together with its accompanying vein, is then clamped along with a part of the capsular fascia which is a loose structure and is readily drawn up. The artery is then ligated proximally to the clamp and the ligature anchored in the fascia. A ligature is also passed around a portion of the remaining fascia in the clamp and this is also anchored if it includes many vessels. The artery and portion of fascia thus ligated are then cut between the ligatures and the hemostat. The assistant should hold the stump with tissue forceps as it is cut and not release it until it is ascertained that there is no bleeding. The fascia remaining attached to the lower portion of the gland is caught up in a hemostatic forceps and fractionally ligated, a small portion being included in each ligature. The importance of rigid hemostasis must not be underrated for it is to be borne in mind that following this operation there is a marked vascular dilatation all over the body and considerable oozing may take place from vessels which at the time of operation appear insignificant. If the capsule is carefully ligated in this manner, we are certain to control a great deal of lymphatic seepage. Care

must be taken when clamping the capsular tissue that the descending branch of the ansa hypoglossi is not included. If raised it may be pushed out of the field by gently wiping with dry gauze. The recurrent laryngeal nerve which supplies the intrinsic muscles of the larynx must not be cut as stridulous breathing may occur to say nothing of the discomfort the animal would incur in the loss of his powers of vocalization. It should be identified and pushed out of the field of operation. Occasionally small vessels pass into the gland from the sternothyroid muscle and these must be securely ligated.

After the inferior pole has been freed it is drawn well up out of the wound. This brings the superior thyroid artery with its branches and the superior thyroid vein into view. The artery gives off a number of small branches close to its origin, the most of which are distributed to the glands, others going to the infrahyoid muscles and larynx. The parathyroid bodies frequently lie in the adventitia of this artery and the surrounding tissue. For these reasons it is necessary to ligate the artery close to its origin. Occasionally the artery is very short, so that there is scarcely room to insert a hemostat between its origin from the carotid and the gland. These are factors that increase the necessity for making ligatures very secure. The largest branch is first clamped and ligated, the capsule is then clamped, a small portion at a time and the vessels held in the clamp ligated and cut. The ligatures must be drawn tight, but not so tight as to cut through. The operator proceeds in this manner from the lower outer portion of the capsule about the superior pole of the upper and inner extremity. It is best to secure the larger vessels in a hemostat before cutting. The hemostat is left on the stump until all the vessels have been cut. This can only be practiced where the artery is long enough to permit of a long stump. At the upper and inner part of this pole several small vessels from the trachea will be encountered (the interior tracheal vessels which are branches from the inferior laryngeal). They are sometimes overlooked and produce considerable bleeding. The ligatures are all strongly anchored in the fascia. Before releasing the stump, it must be seized with the tissue forceps and carefully sponged and inspected for any oozing. If care has been observed to include every bit of connective tissues at the superior pole between the ligatures, there will be no bleeding. In this manner the gland is dissected out from below upward and everything is ligated as it is cut. We proceed in the same manner with the gland on the opposite side. After both lobes are removed the region is carefully inspected for accessory lobes. When any are found they are removed.

The wound is closed by three rows of sutures. The sternohyoid muscles are approximated by three interrupted sutures placed equal distances apart. We used a small curved needle without a cutting edge for sewing these muscles. A row of continuous sutures is then put in the platysma fascia. The skin is closed with interrupted sutures. No drainage is used and if the technic has been properly observed there will be little tumefaction and little discharge. The dressing consists of dry gauze harnessed about the neck and shoulders. This is applied during the recovery from the anesthetic.

I shall briefly mention some of the measures used in preventing shock. Kindliness is very essential. In most cases we find that by gaining the confidence of the animal we are able to place it on the board and shave and cleanse

the field of operation before beginning the anesthesia. This alone considerably shortens the time of anesthesia. Ether or ether chloroform and alcohol mixture was used. The anesthetic is begun very gradually. The mask is first placed on the head without the anesthetic, when the animal is quiet and not frightened. The animal is allowed to toss the mask off a few times until it becomes reassured and then a few drops of ether are administered. If force has been avoided up to this point, the animal will breathe the anesthetic without much persuasion. As he feels the influence the rate of administration is gradually increased. In this way, many animals have been carried over to complete anesthesia, practically avoiding the excitement stage.

After recovery from the anesthetic the animal is wrapped in warm blankets which are changed every 5 or 10 minutes. The room is kept warm and the cage scrupulously clean. Because of the tendency towards vomiting after removal of the parathyroid glands solid food is not given. Plenty of fresh water was supplied and this was loaded with lactic acid bacilli with the hopes that their presence in the intestines would prevent the production of toxic amines formed by decarboxylation of amino acids. Animals showing signs of infection or hemorrhage were discarded.

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FURTHER STUDIES IN NICOTINE TOLERANCE*

BY CHARLES W. EDMUNDS, M.D., AND MAURICE I. SMITH, M.D., ANN ARBOR, MICH.

THE question as to how the body acquires the condition of insusceptibility to the effects produced by the introduction of various poisons is one which has always excited a good deal of interest from both the scientific and from the practical standpoints. That such an insusceptibility is developed is well recognized by everyone, but the mechanism by which it is formed is in many cases quite obscure, while in the case of other drugs recent investigations have shed a good deal of light upon the question.

It is unnecessary to enter into a lengthy discussion of the matter in this place as there have been many papers dealing with different phases of the matter, and recently an address by Dixon¹ gave a summary of much of our knowledge upon this subject. In this address, Dixon after discussing the explana-

*From the Pharmacological Laboratory of the University of Michigan.

¹Dixon: Proc. Royal Soc. of Med., v, 1911, 1.

tions which have been advanced as to the mechanism of the tolerance which is gained by the body to the effects of many of the poisons, dwells at greatest length upon the question of nicotine tolerance. This general consideration of nicotine was supplemented the following year² by a detailed account of experimental work which he had carried out in the University of Cambridge in collaboration with W. E. Lee. As a result of the experiments outlined in this paper the writers draw the conclusion that nicotine tolerance is produced by an increase in the ability of the body tissues to destroy the poison.

Before taking up the experimental evidence in support of this view, it may be well to discuss briefly a few of the points raised by Dixon and Lee in regard to earlier work along this line and especially those criticisms which they offer in regard to the experiments which one of us³ carried out some time ago upon this subject.

These points are not brought up with any desire to enter into a controversy but in order to clear up any doubts which might have been raised in regard to the work of others as well as to the particular paper mentioned above.

In the first place, Dixon and Lee explain the failure of Gouget, Adler and Hensel and many of the earlier workers to obtain tolerance to nicotine in animals by the fact that they gave the nicotine by intravenous or subcutaneous injection and they add that "tolerance either cannot be obtained or is insignificant when such methods are adopted." This statement may be true but if so why do Dixon and Lee adopt these methods themselves when they desire to induce tolerance in animals? And not only do they adopt them but in their description of the effects of these intravenous and subcutaneous injections they say that the effects of the "later doses were a little less pronounced than the earlier." Was this diminution in effects due to tolerance? It would be quite possible were it not for the statement quoted above that the methods of administration employed are practically useless for this purpose. Moreover these animals which were rendered tolerant by methods which are condemned are used to demonstrate the mechanism of the production of tolerance. The only conclusion which seems justifiable is either that these animals were not tolerant or that the criticism of the work of Gouget and others is not correct.

Another point which may be considered is the test of tolerance which should be employed. Dixon and Lee do not consider vomiting a valid test, but at the same time it is rather hard to tell just what standard they selected. In the earlier subcutaneous administrations their rabbits showed tremors, twitchings, and signs of paralysis, while after intravenous injections they had convulsive movements and complete unconsciousness, all of which after the later doses are said to have appeared less pronounced than the earlier ones. Such a standard, however, lays itself open to the very serious criticism of being entirely too indefinite and as being too much a subject of opinion. Of especial interest in this connection is the testimony of Gouget who found that the intravenous injection of the infusion of tobacco into rabbits was always followed by convulsions which were always about the same where equal doses were given.

In addition to Gouget, Adler and Hensel report that nicotine in 1½ mg.

²Dixon and Lee: *Quart. Jour. Exp. Physiol.*, v, 1912, 373.

³Edmunds: *Jour. Pharm. and Exp. Therap.*, 1, 1909, 1.

doses, injected into the ear veins of rabbits produced convulsions which were always of the same intensity whether ten or a hundred injections had been given.

And this condition is exactly what would be expected if the nicotine tolerance is gained by an increased destruction of the alkaloid, as in intravenous injections no time would be allowed for such destruction.

Coming back, therefore, to the question as to whether the production of vomiting following subcutaneous injection can be considered a valid test, it may be said that that is perhaps a matter of opinion. It has the advantage of being perfectly definite and of equal importance it is the one symptom in man which denotes the possession of tolerance. The tyro in the use of nicotine rapidly shows his susceptibility to the drug by nausea and emesis and the fact that he has gained tolerance is shown later by the absence of these symptoms. Some individuals never gain tolerance as is shown by the fact that the use of tobacco is always accompanied by nausea. It would seem, therefore, that the use of this test for animals is perfectly logical and reasonable.

It is, of course, true as Dixon suggests that in case the drug is given by the stomach local irritation may produce vomiting, but a study of details of experiments I and II in the earlier paper referred to would convince anyone that local irritation was not the cause of the vomiting in the experiments. In Experiment I, for instance, with the doses being given under exactly the same conditions from day to day, there was what appeared to be a gradually increasing susceptibility to the drug so that whereas the cat had early tolerated 15 mg. doses of nicotine chloride it later vomited from doses of less than one-half that amount.

Finally, as a minor objection these writers appear to doubt whether any tolerance whatsoever was really obtained in the experiments on dogs in which large doses of nicotine were injected. They suggest that the failure to vomit after small doses is due to the depression which would follow the stimulation produced by the large toxic doses. This, of course, is a very important suggestion because if it is justified it would indicate the entire failure of the experiments with resulting erroneous conclusions. Here again, a careful reading of the paper and a study of the protocols to Experiments V and VI will show that such a condition as suggested was taken into consideration and that to avoid the effects of one dose being active at the time of the next dose, the injections were frequently made at intervals of two or three days but by experience it was found that this was not necessary "as the animals appeared to vomit just as frequently after a three or four day interval as after a rest of only one or two days."

In Experiment V one instance may be mentioned as illustrating this point. Fifteen mg. doses of nicotine were being given to a dog which had originally vomited after 12 mg. doses. On April 17th 15 mg. caused vomiting and on April 20th, that is three days later, 15 mgs. again caused vomiting but on the 22nd it caused no vomiting. Again, on the 24th, 27th, and 29th of the month, that is at either two or three day intervals, 15 mg. produced vomiting. We may repeat what was said in the original paper that the only conclusion that could be drawn from a study of the numerous injections made was that it seemed to make no difference in the results whether the injections were made at intervals of one or two days, or at intervals of three or four.

But granting that the criticism of Dixon and Lee in regard to the failure to vomit is valid, did not they, even after perceiving the supposed error, fall into the same error themselves? Their rabbits were injected on alternate days, and may not the apparent lessening of symptoms in their animals also be due to lack of recovery from previous doses? If it was possible in our series, it was equally possible in theirs.

Also the doses of nicotine which they gave were quite as large proportionately as those which were given to the dogs in the earlier work. In only one of the dogs was a 50 mg. dose exceeded and this for only four injections and such a dose of nicotine chloride for a hound weighing 15 Kg.⁴ is not larger than a dose of 5 mg. of the alkaloid nicotine for a rabbit weighing less than one kilo. The Dixon and Lee paper also speaks of the lack of importance of the effects of "enormous doses used in the Edmunds experiments," but a little calculation will show that the average dose in Experiment V in the early part of the work was about 2 mg. of the nicotine salt (equal to 1.4 mg. nicotine) per Kg. and in only three injections was 4 mg. (2.8 mg. nicotine) per Kg. body weight exceeded, while Dixon and Lee's small doses were in the only protocol given 5 mg. of the alkaloid nicotine for a rabbit weighing 945 G. It is certainly true also that not one of the dogs showed symptoms anything like as severe as those which Dixon and Lee describe. Their rabbits showed twitchings, tremors, partial paralysis, and complete unconsciousness, while none of the dogs showed symptoms more severe than vomiting, trembling, and in some cases weakness in the hind legs. If, therefore, the "absence of vomiting" in the dogs does not indicate tolerance, as they suggest, neither do the effects which "appeared less pronounced" in their rabbits and, therefore, their rabbits were not tolerant and the whole theory of the mode of acquiring tolerance which they worked out must naturally fall to the ground. Such can be the only logical conclusion.

After this preliminary discussion which will serve the purpose of clearing up questions in regard to the reliability of previous work, the general content of the Dixon and Lee paper may be briefly summarized, taking for granted that the rabbits were rendered tolerant. After this had been accomplished by means of three subcutaneous and eleven intravenous injections of 5 mg. nicotine, the tolerant animal and a control were killed, and portions of the livers, freed from blood, were ground in a mortar with sand and saline solution and to this were added 2 c.c. of a one per cent solution of nicotine and 1 c.c. tuluol and the whole incubated for two and one-half hours. The solutions were then acidified, boiled, neutralized and filtered through muslin and paper, the two filtrates being of equal amount. The relative content of nicotine in the solutions was then estimated by means of the blood pressure rise which was produced in cats when equal doses were injected into the veins. Judged by this standard it was found that the liver of the animal which had been injected with nicotine destroyed a considerably larger amount of nicotine than did the liver of the normal animal. The writers further conclude that this destruction is brought about by the action of a ferment which is developed in the tissues of the rabbits under the influence of the injected drug.

⁴The shepherd dog used in Experiment IV of the early series weighed 15.6 Kg.; the black and white dog used in Experiment VI weighed 14.2 Kg.; and the hound used in Experiment V was about the same size.

These results are very interesting and important as they seem to indicate the mode by which the body protects itself when it is subjected to the influence of nicotine over a considerable period of time. It seemed to us therefore that inasmuch as it had been shown that it was possible to develop in dogs some degree of tolerance to the drug it would be of great interest to see whether in these animals it would be possible to demonstrate a mechanism of acquiring this tolerance similar to that described by Dixon and Lee for rabbits.

The method used in the effort to gain the tolerance was the same that was utilized in the earlier work, viz., to make the injections subcutaneously and starting in with a small dose to rapidly increase the dose until fairly large amounts were being given. The injections which were made at intervals of two or three days in the form of nicotine chloride, averaged in number from fifteen to twenty injections for each dog. It is unnecessary to give protocols for all the dogs which were treated, so only a condensed table will be given later in the article. It may be well to say that in no case were the symptoms any more severe than those described in the earlier paper. With the smaller doses the usual salivation and vomiting were present, accompanied in the case of the larger doses with dyspnea and some weakness in the extremities which was probably secondary to the repeated vomiting.

Ten dogs in all were used for this part of the work and the estimation of the nicotine destroying power of each liver was carried out upon cats in the same way as had been done by Dixon and Lee. After the dog had been killed, its liver was removed, freed from blood as completely as possible, and fifty grams of liver substance were placed with some sand in a mortar and to this were added 50 c.c. saline solution, 1 c.c. of toluol and 40 mg. of nicotine in the form of hydrochloride. After these had been ground up thoroughly the extract was incubated at 38° for three hours, then acidified, boiled, neutralized, strained and filtered. Fifty grams of liver substance from a normal untreated dog was treated in precisely the same manner, the two extracts being carried through at the same time.

When these extracts were completed they were used for injection into cats which were prepared for blood pressure estimation in the usual way. After being anesthetized with chloretone, cannulas were inserted into the trachea, into one of the carotid arteries and into the two external jugular veins. During the course of these estimations each animal was kept warm by means of an electric heating pad and given artificial respiration with heated air. The injections which were given at intervals of six minutes were given alternately, first the extract from the tolerant animal into one jugular followed by the control into the vein of the other side. The alternating doses were then continued until such relative amounts were found that the rise from each extract was alike. In some cases these doses were then doubled in order to confirm the ratio established by the smaller doses.

The following table will give the most important details of the experiments:

No. of Exp.	Wt. of Dog.	No. of Inject.	Initial dose of Nicotine given in form of the chloride.	Largest dose of Nicotine.	Relative strength of extracts as determined by effect on blood pressure.
1	10.9Kg.	16	5 mg.	50 mg.	1 c.c. tolerant—2 c.c. Control
5	6.6	14	2.5	50	1 c.c. " —0.5 c.c. "
6	7.5	18	2.5	53	1 c.c. " —0.75 c.c. "
7	8	17	26.5	53	1 c.c. " —1.4 c.c. "
8	14	17	26.5	58	1 c.c. " —1 c.c. "
9	15	15	26.5	58	1 c.c. " —1 c.c. "
10	7	23	13.3	57	1 c.c. " —1 c.c. "
12	8	17	13.3	58	1 c.c. " —1 c.c. "
13	7	12	13.3	46	1 c.c. " —1.15 c.c. "
14	6	20	13.3	58	1 c.c. " —0.75 c.c. "

A survey of this table shows first that out of the ten dogs which were subjected to ascending doses of nicotine in only three was there any evidence of an increased power of destruction of the alkaloid over that displayed by normal animals. These animals were Numbers five, six and fourteen. In them it seemed that the facts shown by Dixon and Lee as being true for the rabbit were also true for dogs. In four more dogs however (Numbers eight, nine, ten and twelve), could no such increased power be shown. Extracts of livers from these animals possessed no more power to destroy nicotine than did those prepared from livers of animals which had received no nicotine.

And as an offset to the first three mentioned there are still another three (Numbers one, seven and thirteen), the livers of which did not display as great destroying power as did those from normal dogs. The net results then would seem to be practically negative. The explanation of our results probably is that the livers of all dogs possess some power of destroying nicotine but that this ability is subject to great variations in different animals. If this power of destruction is due to a ferment action, the ferment must be increased in amount only very slowly and with great difficulty. That it is probably a ferment action is suggested by one of our experiments (No. 6) when a portion of the liver was boiled before being incubated—all the other particulars of the technique being the same as in the other cases—the results were as follows: 1 c.c. control—1.4 c.c. unboiled extract of tolerant—0.75 c.c. boiled tolerant. The ferment having been destroyed, the solution was relatively stronger even than the control which in itself had destroyed a certain amount of nicotine.

That some normal untreated livers are not devoid of the power of destroying nicotine is further shown by some of the experiments in which the control liver extract after having been compared with the tolerant, was compared with a nicotine solution of equivalent strength. Three experiments tend to show that the normal liver extract after incubation contained less nicotine than a control nicotine solution. Thus in one experiment (V) 1 c.c. of the normal control liver extract gave a smaller rise of blood pressure than .8 c.c. of the nicotine solution. In two more experiments (VI and XIV) 1 c.c. of the normal (control) liver extract gave rise in blood pressure equivalent to those produced by .75 c.c. and .67 c.c. of nicotine solution respectively.

In connection with our results it is interesting to note that Dixon and Lee

say that in three of their rabbits they failed to find any evidence of tolerance so it appears that the two animals do not differ very greatly in this respect.

The conclusions we would draw from our work are that the livers of all dogs possess some power to destroy nicotine and that the destroying agent is probably a ferment. It is possible that this ferment may be increased in quantity by the constant use of the alkaloid but our experiments do not furnish any support for this theory. The main difficulty appears to be that under normal conditions the destroying powers of different livers vary so much in strength that it is almost impossible to state that it has been increased by treatment.

HEXAMETHYLENAMINE AS A URATE SOLVENT, AND DIURETIC, AND ITS EFFECT ON THE REACTION OF URINE*

BY PAUL J. HANZLIK, M.D., CLEVELAND, OHIO.

A LONG with piperazine and some other methylene diamines hexamethylenamine (Bardet, 1894; Nicolaier, 1894) was originally proposed as a uric acid solvent. This alleged virtue Nicolaier claims to have observed quite incidentally. He noticed that in certain urines to which formaldehyde was added as a preservative the deposits which had formed on standing disappeared, even in the presence of hydrochloric acid. This he attributed to the formaldehyde. Therapeutic possibilities were at once suggested, and hexamethylenamine as a non-irritating formaldehyde compound was proposed as the ideal drug. Its antiseptic properties were also soon recognized, and these have continued to claim a deservedly greater popularity (see Hanzlik and Collins, 1913, and appended literature). However, a considerable medical clientele appear still to possess faith in its supposed virtues as a urate solvent. This is supported and fostered by interested manufacturers, whose exaggerated claims, however, have served to dampen the enthusiasm for these hoped-for virtues.

The mode of action of hexamethylenamine as a urate solvent has never been quite definitely understood. In fact it has been vaguely attributed sometimes to its chemical properties, sometimes to certain alleged physiological effects. For instance, owing to the fact that formaldehyde can combine with uric acid to form soluble compounds, and since hexamethylenamine was supposed to liberate formaldehyde in the body, this has been a favorite explanation with many. Then again hexamethylenamine and uric acid are said to combine directly with the formation of a salt, called hexamethylenamine urate. Finally, diuresis due to some supposed physiological action of hexamethylenamine, also claims its adherents. There are reasons for supposing that neither one of these explanations is necessarily correct, or quite adequate in view of other attending circumstances. A review of the important literature was purposely made with the object of ascertaining what claims, if any, can be made for hexamethylenamine as a urate solvent.

*From the Pharmacological Laboratory, Medical School, Western Reserve University.

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The results of this search are sufficient to indicate the negative value of hexamethylenamine in this direction. Unfortunately the nature of such studies as here reported is surmounted with numerous difficulties, and it cannot be said that the investigations undertaken to elucidate these have been always characterized by the most careful scientific scrutiny. Much of the confusion has resulted from a failure to realize and appreciate the chemical and solubility characteristics of uric acid and its salts and their behavior in the organism. Since these fundamental considerations cannot be safely overlooked in judging of the real value of hexamethylenamine as a urate solvent, it was deemed necessary to present first a resumé of them. Following this the effects of hexamethylenamine will be considered.

URIC ACID AND ITS SALTS.*

Uric acid is a weak dibasic acid and can form three series of salts: (1) neutral or dibasic, normal or secondary urates; these are formed only by strong alkalies and cannot occur under natural conditions, being decomposed by carbon dioxide and even water; (2) acid urates, or monobasic, primary or biurate; these are neutral to litmus; (3) quadriurate; hemibasic or tetra urate, a mixture of uric acid and acid urate (Kohler, 1911); according to Ringer and Schmutzer (1912) a "solid solution of uric acid in acid urate which can be formed at body temperature, but which is unstable on cooling and thus separates uric acid."

Solubility.—Free uric acid is very little soluble in water. According to His and Paul (1900) pure water dissolves at 18° C. only 25 mgms. per liter=1:40,000; at 40° the solubility is doubled. Alkalies, even sodium bicarbonate and phosphate, transform part of the uric acid into acid urate and therefore increase its solubility. Free acids lessen solubility since they decrease the ionization (His and Paul, 1900). Addition of free uric acid to urine may precipitate a part of uric acid originally in solution (Voit) presumably by acting as a crystallizing nucleus. The solubility in serum is much greater (500 mgms. per liter) than in water (Bechhold and Ziegler, 1909). This is probably due to the colloids and it is probable that the colloid constituents of urine also contribute to the solubility of uric acid and urates (Lichtwitz, 1910). Lichtwitz, however, denies an important role to urochrome as was claimed by Klemperer, and finds that practically all the uric acid of urine is in true solution (not colloidal) for it dialyzes easily from urine. Ruedel (1892) claimed urea to have an important solvent action, but Ritter (1897) finds this very limited. The acid sodium phosphate precipitates and liberates uric acid. This is counteracted by the presence of the alkaline phosphate, and is of interest in connection with urine.

According to Allan and Bensch (1848), the solubility of the different urates in water is about as follows: lithium 1:350; potassium 1:800; sodium 1:1200; and ammonium 1:1600; alkaline earths 1:4000 to 6000. Excess of alkali or other salts decreases the solubility by "salting out" in proportion to their concentration (Roberts, 1896; Ritter, 1897). The salts of plasma reduce the solubility to 1/10 of that in water (His and Paul, 1900). Solid sodium acid urate occurs in two forms; when freshly precipitated is gelatinous, colloidal or amorphous, later becomes crystalline. The colloidal form is more easily soluble (Bensch, 1845). Gudzent (1909) claims that two forms exist, the more soluble lactam and the less (2/3) soluble lactim.

Occurrence in the Body and Tissues.—Uric acid as such does not exist in the blood. It appears in the form of the acid urates in solution in the blood and tissues and as solid biurate deposits in gouty tophi (Ritter, 1897). The free acid and acid urate appear in scanty or acid urine. Being crystalline the urate deposits act as powerful mechanical irritants producing some of the local phenomena of gout. These can be simulated by the subcutaneous injection of suspensions of acid sodium urate and in birds by subcutaneous injection of chromates or ligation of the ureters. The cause of the abnormal appearance of urate deposits in gout is not understood. Recently, Benedict (1915) has obtained results with ox blood which seem to indicate that uric acid appears in some combined non-excretable form in the blood corpuscles, besides the urate in the plasma, which would be the excretable form.

*The material in small print under this heading was in part abstracted from the manuscript of Professor Sollmann's revised Textbook of Pharmacology.

Effects of Solvents on Urate Deposition in Tissues.—The mobilization or removal of urates from the tissues in such conditions as gout, and possibly others, would be desirable; also, to prevent the formation of urate deposits in tissues and urine. What has been said concerning the chemical properties of the urates serves to indicate the difficulties of doing this.

The study of solubility is greatly complicated by small quantities going into solution, polyvalence, formation of colloidal solutions, supersaturation and the influence of small quantities of impurities, decompositions, etc. (His and Paul, 1900). Alkalies dissolve a limited amount of free uric acid by conversion into the more soluble acid urates and that limited success is confined to urine.

When all the uric acid exists as acid urate, as it does within the body, the addition of alkali can have no further solvent action. On the contrary, excess of alkali or other salts tends rather to decrease the solubility of the urates. It is evident, therefore, that no direct solvent action can occur in urate tophi (Ritter, 1897). This inefficiency has been confirmed clinically. If alkalies have any effect, which is doubtful, it would be solely by their diuretic action. Loghem (1907) claimed that hydrochloric acid is more useful for preventing formation of urate deposits in animals, presumably by lessening the "salting out." Ritter (1897) had a similar idea, but this was not confirmed by Staal (1908).

The conditions for solvent efficiency of alkalies is much better in acid urine. This contains not only acid urates, but also free uric acid often in saturated solution. When urine cools, it becomes more acid and under other conditions solutions may become supersaturated. The uric acid will then precipitate alone or in combination with a part of the acid urate (quadriurate). This can be prevented either by making urine more dilute (diuresis) or by making it less acid. Alkalies according to Buchheim (1853) act in both directions. Too high concentrations of alkali as other salts render acid urate less soluble, but in practice the administration of alkalies does not raise the total concentration of urine especially if the water is taken freely. Alkaline urines would also dissolve actually precipitated uric acid, for the urine, after the administration of alkalies, is able to dissolve more uric acid than before (Haskins, 1914). With the practical limits of the alkalinity of the urine, however, the solution is so slight that it has little effect on formed calculi. The introduction of alkaline solutions into the bladder has not been more successful (Buchheim, 1853).

It is sometimes stated that the solubility of urates is improved by the administration of potassium or lithium salts. This is true of pure solutions, or when sodium urate (or urate stones or tophi are treated with lithium carbonate), but it cannot occur in the presence of an excess of sodium; for according to ordinary chemical laws, when several bases compete for an acid, it is the least soluble salt that is formed. Since there is always an excess of sodium ions in the blood and urine, the lithium and potassium would have no chance to exert their solvent action. The same reasoning applies to certain organic bases, such as the ethylen-amines (also, hexamethylenamine), piperazine and piperidine. These also would be unable to compete against the sodium. Moreover they are excreted imperfectly, so that their efficiency in the urine is probably inferior to that of ordinary alkalies (Haskins, 1914).

The relation of reaction and volume of urine to solubility of uric acid was studied by Blatherwick (1914). He found that the extent of solution of uric acid in urine appears to be chiefly a function of hydrogen-ion concentration. There was a striking regularity in the deposition of uric acid in urines having a greater hydrogen-ion concentration than 7.0 (neutrality), while alkaline urines showed an increased capacity to dissolve. Very acid urines were generally found to be supersaturated and neutral specimens were nearly saturated. Ordinary variations in urinary volumes exerted an insignificant influence on uric acid solubility. These changes can be effected by appropriate variations in a dietary composed of commonly used articles.

EXCRETION OF URIC ACID (ASIDE FROM DIETARY INFLUENCE).

The various factors in the quantitative excretion of uric acid by the kidney, using the accurate colorimetric method of Folin, has been studied by Robertson (1914) with no definite results. The injection of pituitary, saline, barium chloride, caffeine and piperazine and sodium sulphate produced no changes in uric acid excretion which were sufficiently independent of urine flow to suggest any specific effect of the agents used. In fact it could not be definitely es-

established whether increased water excretion or change in the blood flow through the kidney is primarily responsible for the parallelism between increase in urine flow and uric acid excretion. Certain of the results indicate that uric acid excretion varies independently of the urine flow, and that increased uric acid excretion does not necessarily lead to much increase in the amount of urine. Preti (1915) has recently shown that the increased intake of carbon dioxide (respiration) increases the elimination of uric acid. So far as drugs are concerned, it can be stated that atophan (Folin and Lyman, 1913; Haskins, 1913), salicylate (Denis, 1915), and colchicum produce a definite increase in the excretion of uric acid into the urine; alcohol is somewhat doubtful (Jackson and Blackfan, 1907). According to Abl (1913) diminution in the excretion is brought about by calcium salts, barium sulphate and bismuth nitrate, whereas mustard, arsenic, colchicine, thorium, sulphur, santonin, glycerin, choline, chloral, neurine, strontium and piperazine increase elimination. These drug actions may be explained by increase in renal permeability in some cases, or disturbances in the systemic circulation in others; increased oxidation may also play a part.

The role of the digestive glands in the production of endogenous uric acid has recently been emphasized by Mendel and Stehle (1915). Drugs which augment glandular activity, such as pilocarpine, brought about an increased excretion, whereas atropin (antagonist) lessened. Mechanical work of the intestine alone did not influence the production of uric acid.

This rather lengthy preliminary survey is considered necessary for a proper appreciation of the properties of uric acid and the urates and the various conditions which influence and underlie their behavior in the body. It will suffice to indicate the complex nature of any problem which pretends to the analysis of the solvent properties and excretory powers of a given agent for uric acid or urates. From what has been said of the influence of the various factors governing the solubility and excretion of urates, and the probable chemical and physiological effects which can be produced in this direction by hexamethylenamine, its chances of success as a uric acid solvent would be rather limited. There are certain properties which hexamethylenamine must exhibit if it is to produce the desired effects. These are: (A) As a urate solvent: (1) a urine containing hexamethylenamine must take up distinctly more uric acid than do normal urines of corresponding acidity and concentration; (2) to be of value in calculi, gravel, and removal of free uric acid, it must dissolve these more readily than urine itself without the influence of concentration and alkali. (B) For urate elimination: Administered internally a definite increase in elimination of urates, uninfluenced by other factors, such as diuresis, diet, drugs, etc., must be demonstrated. The available facts in the literature will now be judged on the basis of these conditions, and unfortunately, it may be intimated, with not always the best results for hexamethylenamine.

ADDITION OF HEXAMETHYLENAMINE TO PURE URATE.

According to the experiments of Tuncliffe (1897) a 3.5% aqueous solution of hexamethylenamine dissolved 0.7% of added uric acid at 17° C.; 3.3% at 36° C., by prolonged heating on a water-bath. The solution of hexamethylenamine was acid to litmus. Hence it is probable that formaldehyde was present, and caused uric acid to go into solution. Casper (1898) found that 300

Gms. of water dissolved 0.024% of uric acid in 5 days at room temperature, none at 37°, whereas a 1.3% hexamethylenamine solution dissolved 0.18% in 5 days at room temperature, and 0.016% at 37° C. Nicolaier (1899) observed that hexamethylenamine solutions dissolve more uric acid than water alone at 18° C., and this was augmented by about seven times at 37° C. According to Tunicliffe and Rosenheim (1898) solutions of uric acid in hexamethylenamine gelatinize on cooling, behaving in this respect like colloids, but differing from them by being able to dialyze. The experiments of Nicolaier (1899) indicate that while hexamethylenamine dissolves uric acid and urates at body temperature, it is less active in this respect than piperazine. Heinz (1907) and Penzoldt (1910) claim that hexamethylenamine *in vitro* has considerable solvent action for uric acid, but no evidence is offered for this. Ortowski (1910) reports that the solvent properties of hexamethylenamine for uric acid at 37.5° C. are slight, but that passage through the body imparts to the urine marked solvent properties. Stevens and May (1911) found that a 1.5% solution of hexamethylenamine was only a fair solvent for added uric acid, requiring considerable agitation to effect solution. Only 0.035% of uric acid was dissolved, and on prolonged standing (1 to 14 days) in contact with hexamethylenamine no destruction or decomposition of the uric acid was demonstrable. As compared with hexamethylenamine the solvent properties of lycetol were less, but that of piperazine greater. Kobert (1906) states that hexamethylenamine *per se* forms a compound with uric acid from which uric acid cannot be regenerated. This does not seem to be the common view. Whatever solubility takes place is said to be due to liberated formaldehyde with the formation of diformaldehyde uric acid (Tollens; Nicolaier). This is believed to be the case by Haskins (1915), whose work shows definitely that hexamethylenamine in concentrations (0.05 to 0.1%) comparable to amounts excreted after therapeutic doses has no demonstrable influence on the solubility of uric acid under conditions resembling urine. In higher concentrations (1%) hexamethylenamine dissolved small, but distinctly demonstrable, quantities of uric acid with the liberation of formaldehyde. The experiments were performed by adding uric acid to mixtures of di- and monosodium phosphates, since the degree of reaction plays an important part in the solubility. Haskins found that 0.1 to 5% solutions of hexamethylenamine in plain distilled water dissolved 0.035% to 0.25%, respectively, of uric acid, but he observed that the acidity (hydrogen-ion concentration) of ordinary distilled water (due to CO₂) is sufficient to cause liberation of formaldehyde from the hexamethylenamine. The solubility, therefore, was not due to hexamethylenamine *per se*. Water alone dissolved only 0.0084% of uric acid. Another instance of how a relatively small degree of acidity tends to facilitate the uric acid solubility of hexamethylenamine as compared with a small degree of alkalinity was observed by Haskins as follows: A 1% solution of the drug in a phosphate mixture of a hydrogen-ion concentration of 7.2 (alkalinity) dissolved a smaller amount of extra uric acid than a 1% solution of the drug in a phosphate mixture of the same concentration, but of greater hydrogen-ion concentration, 6.8 (acidity).

Why hexamethylenamine dissolves extra uric acid in alkaline mixtures is explained by Haskins (1915) as due to the fact that the acidity of the solution gradually changes as uric acid is taken up. For instance, the filtrate from

a 7.2 (alkaline) phosphate mixture after shaking with uric acid had an acidity of 6.8. In consequence of this, formaldehyde was finally liberated in the hexamethylenamine phosphate mixture and dissolved extra uric acid, whereas in the same acid solution (6.8) formaldehyde was being liberated during the entire time of shaking. While the time for liberation of the formaldehyde may be relatively short, this is compensated for by the greater concentration of the drug.

Concerning the combining power of formaldehyde with uric acid, it may be further said that formaldehyde combines with uric acid to form loose and in part at least, very easily soluble compounds in water. This was investigated by Weber, Pott, and Tollens (1897), but it is not quite clear (although conceivable) whether this combination takes place under conditions of the body (i. e., in urine) when formaldehyde is liberated from hexamethylenamine. These investigators prepared formaldehyde compounds by adding uric acid to 40% formaldehyde at 100 to 110° C. Two sets of products, which differed in elemental composition, were obtained, and formaldehyde from one of these (di-formaldehyde) was liberated by prolonged boiling only.

In Nicolaier's (1906) contribution on compounds of uric acid with formaldehyde, nothing of importance is added to the subject. In their work both Tollens and Nicolaier make use of 40% formaldehyde in the preparation of these uric acid compounds. In no case have there been used such concentrations of formaldehyde as can appear in the urine after administration of hexamethylenamine. Final judgment as to the possibilities of formation of such formaldehyde uric acid compounds must be deferred until these have been investigated under conditions of the body.

The degree of the reaction (H^+ conc.) is the most important factor in the problem of urate solubility and, unfortunately, was practically entirely overlooked by all workers previous to Haskins (1915). This greatly limits the value of their work. As will be seen later, it is the paramount factor of importance in urinary solubility, which will shortly be considered.

The nature of the evidence just cited permits us to conclude that the solubility influence of hexamethylenamine *per se* when added directly to pure uric acid is practically nil; particularly if the influence of reaction, salts, temperature, and other factors are discounted. When formaldehyde is liberated, a small but practically insignificant quantity of uric acid is dissolved.

ADDITION OF HEXAMETHYLENAMINE TO URATE CALCULI.

The observations on this are extremely limited. According to Nicolaier (1904), the action of hexamethylenamine on uric acid concretions is slow and difficult to judge. His own observations are limited to urine, and will be discussed later. Casper (1897) states that hexamethylenamine solutions, no matter how strong, hardly dissolve uric acid calculi any more than ordinary water, and at room temperature solution proceeds somewhat more slowly than at body temperature. This is confirmative of some preliminary experiments reported by Loebisch (1897). Casper also observed that as a solvent for calculi, hexamethylenamine is no more effective than lysidine or piperazine or other agents recommended for that purpose. Drake-Brockmann (1900) found that weak and concentrated solutions of hexamethylenamine produced no change in the weight of small portions of immersed calculi even after incubation for several days.

While the paucity of data must be admitted, the general tone of the existing observations is unfavorable to hexamethylenamine as a solvent for calculi.

SOLVENT ACTION OF HEXAMETHYLENAMINE URINES ON ADDED URIC ACID AND URATE.

Nicolaier (1899) observed that urines which previously did not dissolve added uric acid at body temperature, did so when they contained hexamethylenamine. However, Nicolaier reports but few results, and the influence of the reaction of the urines was not studied. Casper (1898) performed a single experiment with 200 grams of urine of a man who received 3 grams of hexamethylenamine, and found that in 5 days at room temperature 0.027% of added uric acid was dissolved, and 0.14% at 37° C., but no reference is made to the reaction of the urine during this time, and Casper, rightfully, does not lay stress on this observation. Tunicliffe and Rosenheim (1898) observed that when hexamethylenamine (0.2 Gm.) was added to urine (100 c.c) kept at body temperature for two and a half hours, about 6 times as much of the added uric acid was dissolved as compared with the same urine without the drug. Here again nothing is stated concerning the reaction or concentration of the urine, and this same criticism applies to the claimed superiority for piperidine and lysidine, which according to these authors would be about thirty times that of hexamethylenamine.

Haskins (1915) found that when hexamethylenamine (0.05% to 0.1%), in quantities comparable to therapeutic, was added to normal urines, no more added uric acid was dissolved than in the same urines without the drug. Strong concentrations (1%) of the drug dissolved more uric acid than the urine itself.

In connection with this the observations of Tunicliffe and Rosenheim (1898) on the solubility of serum for sodium biurate may be mentioned, for, so far as I know, they represent the only data of their kind in the literature. Tunicliffe and Rosenheim found that when hexamethylenamine (0.1 Gm.) was added to beef serum (100 c.c.) containing sodium biurate, the solubility of the biurate was increased over that of serum alone. With serum alone, the solubility was 1:60,000. When hexamethylenamine was added, this was increased to 1:14,000. If the solubility of biurate in serum alone is given, the value of 1 of that serum plus hexamethylenamine would have the value of 4.5. Piperidine gave 4.7, but lysidine and piperazine less than hexamethylenamine. The quantity of biurate in the serum originally present was very small, about 0.00029%. Although the reaction of the serum was not observed, these experiments appear to have been otherwise properly performed. If the serum possessed a neutral or very slightly alkaline reaction (even after incubation), then it appears that hexamethylenamine itself would possess some solvent influence on biurate, which, although an acid salt, would not influence the reaction sufficiently. On the other hand, if the serum solution became truly acid, as it frequently the case with incubated body fluids, then formaldehyde was liberated and would explain the solubility to some extent at least. A confirmation of these experiments is necessary before this feature of urate solubility by hexamethylenamine is accepted as proven.

EFFECT OF ADMINISTRATION OF HEXAMETHYLENAMINE ON URIC ACID AND URATE SOLUBILITIES.

Nicolaier (1895) claimed that an individual passing a urine which on incubation and on standing deposits urates, will not show this if hexamethylenamine

mine is given in sufficient dosage, and that the urine loses its uric acid solubility when the administration of hexamethylenamine is stopped. A case of leukemia is cited in which the urine exhibited urate sediments on all days except when hexamethylenamine was administered. The dose of hexamethylenamine was 6 Gms. per day. This effect was not thought to be due to increase in diuresis, since dilution of the same urine did not alter the quantity of sediment; also not to concentration, since the specific gravity was the same. The reaction of the urine was said to be unchanged qualitatively (acid), but no observations on the reaction in a quantitative manner were made, nor on the quantity of uric acid. In fact solution of uric acid in the incubated hexamethylenamine urine progressed slowly for several days; this being especially true of urate concretions.

Smaller daily doses of hexamethylenamine (1 to 1.5 Gms.) also caused urine to take up added uric acid. However, this varied within wide limits, and was greatest usually with the least concentrated urines, especially if diuresis increased during the administration of the drug. Nicolaier states that the reaction (acid) was not changed during the medication, however the degree of the acidity from time to time was not ascertained.

Nicolaier states that urine at body temperature could not dissolve concretions, but after sufficiently large doses of hexamethylenamine (1 Gm. three times a day), the urines at 37° began to dissolve the concretions during the first twenty-four hours, and solubility progressed slowly, so that in a few days only the organic protein-like structure remained undissolved. The solution property was lost when hexamethylenamine was stopped. A case from such a series of observations is cited. The degree of acidity of urine was not determined, and diuresis is excluded on the grounds that the same urine, when diluted and incubated at 37°, did not dissolve the concretion.

In his attempt to explain the supposed hexamethylenamine solubility of urate, Nicolaier admits the importance of diuresis. However, this is not thought to be the only cause, since solubility of uric acid occurs without it. Reaction of the urine is considered important in creating a favorable condition for solution. However, since the reaction remains acid and practically unchanged during hexamethylenamine medication, and hexamethylenamine itself is a weak base, and cannot lower the acidity, Nicolaier thinks that too much stress should not be laid on this, and prefers to regard solubility as an action of the drug itself.

The mechanism of this action is attributed to liberated formaldehyde, which is said to form very soluble compounds with uric acid, this being more true at body than room temperature, since heat facilitates the decomposition of hexamethylenamine. No experiment of his own is cited by Nicolaier for this theory. The presence of formaldehyde was not demonstrated, nor is the possibility of the likely quantity necessary for union with the added uric acid indicated.

Casper (1897) states, that after numerous unsuccessful experiments with other reagents, he does not believe the favorable reports of Nicolaier on the solubility of hexamethylenamine for concrements. Casper gave hexamethylenamine in large doses to patients with uric acid, gravel or stone, without observing any positive evidence of the solubility of the concretions. Casper observed that urine during and after administration of hexamethylenamine dis-

solved uric acid just as poorly as urine of the same subject without the drug.

The supposed beneficial results in a case of pyelitis calculus urica on a purin-free diet reported by Loebisch (1897) are attributed to diuresis and formaldehyde liberated in the blood. However, Loebisch falls into error regarding the presence of free formaldehyde in blood. That such does not occur is universally admitted (see Hanzlik and Collins, 1913; McGuigan, 1914). He also states that hexamethylenamine dissolves sodium acid urate at room temperature very slowly, but very rapidly on incubation.

Nicolaier (1904) asserts that the urate solvent property of hexamethylenamine is not due to the drug itself, but rather to the liberated formaldehyde. Nicolaier states again that 1 to 1.5 Gm. of hexamethylenamine has uric acid solvent properties, especially in urate concretions.

The observations of Rosenfeld and Orgler (1896) on the effect of administration of hexamethylenamine are too few and the method for estimation of uric acid unreliable. Their conclusions, that hexamethylenamine acts by lessening formation and improving solubility of uric acid, are unjustifiable, since the results can be explained on the basis of differences in diuresis and sudden changes in diet, which during the experimental observations (when hexamethylenamine was administered) consisted of calves' sweetbread (500 Gm.).

According to Ortowski (1900) very marked uric acid solvent properties are exhibited by the urine only after passage of hexamethylenamine through the body, the precipitability of uric acid is lessened, and in this as well as direct solubility, it is superior to piperazine, uricedine, lysidine and bicarbonate. However, Ortowski's observations are extremely limited, and the results do not warrant the conclusion. The reaction and concentration of the urine were not taken into account. Rosenfeld and Orgler (1896) claim that hexamethylenamine not only increases the solvent properties of urine, but sometimes lessens the excretion of uric acid. Klemperer (1904) claims to have confirmed an observation of Nicolaier that after the administration of 6 grams of hexamethylenamine the 24-hour urine had 1/5 of the entire amount of uric acid present in the form of non-precipitable formaldehyde compound.

Haskins (1915) made a large number of observations, with strict control of the reaction of the urines. Since he is the only investigator who has taken the necessary precaution, the value of his results outweighs all the others. He found that urines after therapeutic doses of hexamethylenamine had no greater solvent power for uric acid than normal urine of similar concentration, or of similar hydrogen-ion concentration. It appeared that when excessive doses (4 grams) of hexamethylenamine were given to normal individuals, the solvent power of the urines was somewhat greater than was secured with normal urines of the same hydrogen-ion concentration. Similar effects, however, were more easily and just as effectively secured by the administration of therapeutic doses of alkaline diuretics and sodium bicarbonate.

From all this it is seen that the older observations on the administration of hexamethylenamine as urate solubility are contradictory. This is due to the fact that such factors as degree of reaction, concentration of the urine, diuresis, dietary influence, the uncertain amount of hexamethylenamine present and formaldehyde liberation were either not, or imperfectly considered. More recent, and better controlled observations which give due consideration to these factors seem to nullify any importance that was attached to the increased solvent properties of hexamethylenamine urines for uric acid above and beyond that of urine itself. An exception must be made to the use of extra large doses of

the drug in which case there appears to be a solvent effect, though slight and practically unimportant, exerted beyond that of the urine itself. It is not absolutely certain that such large doses of hexamethylenamine are devoid of harmful effects. Moreover, by means of alkaline diuretics, in ordinary therapeutic quantities, just as efficient urate solvent properties are imparted to urine. The use of these is also more economical.

EFFECT OF HEXAMETHYLENAMINE ON DIURESIS.

Aside from statements of opinion or impression, I have found no scientific observations on this in the literature. The following are cited for whatever worth they may possess.

Increased diuresis following the administration of hexamethylenamine is claimed by Nicolaier (1895; 1904), Lilienthal (1900), Flexner (1895), Impens (cit. Nicolaier, 1904), and Seifert and Müller (cit. Nicolaier, 1904). Nicolaier claims that 1 Gm. doses were diuretic. In order to obtain efficient diuresis Lilienthal recommended as much water for the patient as could be taken comfortably. Seifert and Müller and Impens state they obtained diuresis with hexamethylenamine citrate ("helmitol"). Nicolaier denies having observed an increase in diuresis after "helmitol," but does not deny the probability of its occurring in some individuals.

Thompson denies the diuretic action of hexamethylenamine; also Strauss and Seibert (cit. Nicolaier, 1904) for "helmitol."

It is evident that nothing definite can be concluded from the sort of evidence available. When no reference is made to fluid intake, no conclusions can be drawn from the urine output. The most that can be said is that the opinions are conflicting.

EFFECT ON THE REACTION OF URINE.

The only scientific evidence available is that reported by Haskins (1915) with the addition of hexamethylenamine to urine. He found that this did not alter the true reaction (hydrogen-ion concentration) of the urine, whether acid or alkaline. After administration of hexamethylenamine Casper observed that the reaction (by titration) of urine remained unchanged. In a study of the effect of different organic bases related to hexamethylenamine on the excretion of uric acid, Haupt (1895) found that the solvent property which the urine possesses appears to be dependent on the alkalinity present, and not on the direct action of the organic base excreted into it.

On the other hand, Cumiston (1898) believes hexamethylenamine lessens acidity. The general tendency of clinical writers has been to claim a change from alkalinity (ammoniacal) to acidity in the reaction. Such claims, and without offering any experimental evidences whatsoever, are made by Nicolaier (1897), Flexner (1895), Greene (1899), Thompson (1899), and Wilcox (1898).

Nicolaier (1897), for instance, makes the statement that urine, after the administration of 1 to 1.5 Gm. of hexamethylenamine, will become ammoniacal at room temperature, but remains clear and retains an acid reaction if incubated at 37° C., and ammoniacal fermentation does not occur even if in a few days a few drops of an ammoniacal urine are added to it. Nicolaier also states that

hexamethylenamine, in doses of 1.5 Gm. per day, rendered ammoniacal urine acid again in a short time. These statements undoubtedly have helped to crystallize the opinions of successive writers on this subject. However, it cannot be said that it has resulted in anything but the perpetuation of faulty evidence.

An example of this is suggested in the statement of Thompson, "It renders a foul-smelling, alkaline urine sweet and acid, and is therefore an acidifier."

In some cases (Flexner, 1895; Greene, 1899), a brief statement endorsing this alleged property is made.

Nicolaier (1904) denies to "helmitol" any influence on the reaction of the urine.

EFFECT ON CALCULI.

Flexner (1895) and Nicolaier (1904) state that calculi are dissolved after administration of hexamethylenamine. Wood (1902) states that hexamethylenamine is less active than piperazine and has no value for calculi. The same is claimed by Casper (1897), who made some observations with the administration of hexamethylenamine. However, these statements are unsupported by scientific evidence. Moreover, they are contradictory, and from other evidence it can be safely stated that the favorable claims reported by some for hexamethylenamine as a calculi solvent are unjustifiable.

SUMMARY.

A resumé of the chemistry and the behavior of various solvents toward uric acid and urates is given, and indicates very remote possibilities of chances of success of so-called urate solvents under conditions of the body.

Urate or uric acid solubility is a matter which is concerned largely with the degree of reaction (hydrogen-ion concentration) and the concentration of fluids. There is no available evidence in the literature to show that hexamethylenamine can influence these favorably.

Recent and reliable evidence shows definitely that hexamethylenamine *per se* in small quantities, or therapeutic doses, imparts to urine no demonstrable urate solvent qualities.

Slight and practically negligible uric acid solvent effects are imparted to urine by excessive doses of hexamethylenamine, and in this, the effects of the common alkaline diuretics are pharmacologically and economically superior.

There is no evidence that hexamethylenamine can dissolve urate calculi.

Regarding the efficiency of the class of drugs called "urate solvents," the statement of Fränkel (1906), being quite to the point and appropriate, may be cited, namely, that no substance has yet been discovered which would form either soluble or easily oxidizable compounds with uric acid under conditions of the body.

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GONOCOCCUS COMPLEMENT FIXATION: A NEW LIPOID ANTIGEN*

BY CARL C. WARDEN, M.D., ANN ARBOR, MICH., AND LOUIS E. SCHMIDT, M.D.,
CHICAGO, ILL.

IN a paper before the Section on Genito-Urinary Diseases at the San Francisco meeting of the American Medical Association, one¹ of us made a preliminary report on the use of the fats of the gonococcus as antigen in gonococcus complement fixation tests, in which the superiority of an alcoholic solution of these fats over watery extracts was shown. This observation grew out of a study² of the biochemistry of the gonococcus, wherein the importance of the lipoid constituents became apparent. This article presents in detail the conclusions summarized in the preliminary report.

ANTIGENS.

In all the tests a good commercial watery antigen (Parke, Davis and Company) was used parallel with the Warden antigen. The latter was prepared in such manner that each 1 c.c. of absolute alcohol contained in solution 0.001 gram of gonococcus fats as fatty acids. To this was added one-third volume of a one per cent alcoholic solution of cholesterol. Alcohol and ethereal extracts of gonococcus substance are of no value as antigen. The gonococcus substance from which the fats were separated was obtained from cultures of twenty different strains. From the standpoint of immunity, the question whether the fatty antigen may not contain a proteid portion, or "rest," of the gonococcus is an important one, as well as that whether the body of the patient may not be sufficiently sensitized to the protein of the cocci present during the disease, but neither need concern us at the present time.

TECHNIC OF THE COMPLEMENT FIXATION TESTS.

We have used the sheep-rabbit-hemolytic system in which the serum was active in dilutions of 1-10000, with fresh guinea-pig complement and thrice washed sheep cells. The titer of the antigens and hemolytic serum was made frequently in the usual manner. The commercial antigen was used in the maximum quantity, permitting complete hemolysis in a given time, and the Warden antigen in one-half the lowest inhibiting dose. The serums and other materials were measured by drops from standard pipettes. When set up, the tubes contained, in addition to the antigens, one drop (0.02 c.c.) of the patient's serum, one drop of complement and sufficient salt solution to equal 1 c.c. After the first incubation of one hour there were added to each tube ten drops (0.2 c.c.) of a five per cent suspension of sheep cells, and an equal quantity of diluted hemolytic serum, representing two units of hemolysin, that is, twice the quantity sufficient to produce complete hemolysis in thirty minutes. The tests were invariably controlled by known positive and negative sera, and separate tubes for each ingredient. Particular caution was used in the titration of the com-

*From the Hygienic Laboratory of the University of Michigan, and the Clinical Laboratory of L. E. Schmidt, Chicago.

¹Warden, C. C.: Vaccine Treatment of Gonorrhea, Jour. Am. Med. Assn., Dec. 11, 1915.

²Warden, C. C.: Studies on the Gonococcus, Jour. Infect. Dis., May, 1916.

mercial antigen as it was observed that individual samples varied with time, and that different lots varied widely in anticomplementary power, although bearing the same unit quantity on the labels. The alcoholic antigen has not varied perceptibly in six months.

RESULTS OF THE TESTS.

The subjoined tables show the outcome of 423 tests upon the sera of 367 persons. Complete inhibition of hemolysis, a positive reaction, is indicated by the sign ++; partial hemolysis, still a positive reaction, by +. Doubtful reactions are shown by ±. We have regarded them as positive reactions as they have been not uncommon in the early and late stages of gonorrhea, and have not appeared in other conditions.

The list includes tests upon the sera of fifty normal individuals of all ages. In no case was the result other than negative. There are included also over one hundred cases of disease other than gonorrhea, in which a certain proportion of positive reactions was obtained, and in which evidence of gonorrhea could not be eliminated, a fact which speaks for the value of the test. Inasmuch as the Wassermann reaction depends on lipoidal properties of the antigen, particular attention was paid to luetic sera, but without detracting from the specificity of the gonococcus antigen.

The divisions made of the material in the tables are purely arbitrary, and have been made merely for convenience in classification.

The presence of gonococcus was determined where possible by culture methods and by *characteristic* smears.¹

The abbreviations P. and W. refer to the commercial antigen and the Warden antigen, respectively.

TABLE I.
DISEASES OTHER THAN GONORRHEA.

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	DISEASE.	DATE.	P.	W.
1.	F. W. L.	39 yrs.	Gc. 1906, Clin. cure.	Colon infect. of kidney.		-	-
2.	J. R. S.	30 yrs.		Syphilis. Wass. +		-	-
3.	J. L.	28 yrs.		"		-	-
4.	E. N.	50 yrs.		"		-	-
5.	A. B. H.	No. 31, 20 yrs.		"		+	+
6.	A. H. (Mrs.)	?		Psoriasis		-	-
7.	S. S. F.	?	Gc. 1903-1912. Clin. cure.	Syphilis. Wass. +		-	-
8.	D. F. M.	34 yrs.	Doubtful. No recent Gc.	Rupture of urethra, 1895.		++	++
9.	B.	33 yrs.	Indefinite smears, 1913.	Syphilis (Wass. +)	5/18/15	++	++
	B.	33 yrs.	" "	" "	5/24/15	++	++
10.	H. H.	39 yrs.		"		-	-

¹The writers do not regard the Gram stain essential. Reliance is placed more upon the appearance of uniformly prepared smears stained with Loeffler's blue, in which the size, morphology and intracellular grouping constitute the characteristic picture.

DISEASES OTHER THAN GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	DISEASE.	DATE.	P.	W.
11.	J. B.	28 yrs.		Pityriasis.		-	-
12.	M. R. H.	1. Female		Anemia.		-	-
13.	M. R. H.	2. "		Gastritis.		-	-
14.	M. R. H.	3. "		Colitis.		-	-
15.	F.			Syphilis-cystitis.		-	-
16.	G. P.	27 yrs.		Hypospadias-cystitis.		-	-
17.	S. Mrs.	22 yrs.	Suspected— husband infected?			-	-
18.	S. baby (daughter).					-	-
19.	H.	30 yrs.		Syphilis.		-	-
20.	G. Mrs.	26 yrs.		"		-	-
21.	P. S.	45 yrs.	Old G. C. hist. Stricture.	Tbc. bladder. (Wass. +)		-	+
22.	E. B. N.	30 yrs.	Old G. C. hist. Clin. cure. ?	Syphilis. Gc. found later.		-	++
23.	J. M.	26 yrs.		Syphilis.		-	-
24.	M. M.			"		-	-
25.	P. Dr.	38 yrs.	Exposure. No discharge.	Gonophobia.		-	-
26.	B. Miss.		2 abortions. Smears.—	Leucorrhea.		-	-
27.	S. G.	27 yrs.	Smears.—	Prostatitis.		-	-
28.	S. Mrs.	?		Syphilis.		-	-
29.	S. C. Mrs.		Smears.—	Leucorrhea; vesical pain.		-	-
30.	F. M.	22 yrs.		Varicocele.		-	-
31.	S. D.			Syphilis.		-	-
32.	R.	45 yrs.	1912 et seq.	Psoriasis.	—8/4.	-	++
33.	B. A. B. H.		?	Chancroid-Bubo.	—8/4.	-	-
34.	R. H. L.	40 yrs.	?	Gout.	— "	-	-
35.	J. K.		None.	Epithelioma.	—	-	-
36.	Case 5. Male.	30 yrs.	"	Asthma.		-	-
37.	S. Mrs.	30 yrs.		Syphilis. Wass.+		-	-
38.	S. child.	5 yrs.		" inherited—		-	-
39.	S. baby.			" " Wass.+		-	-
40.	B. Mrs.	?		General Exam.		-	-
41.	W.	45 yrs.		Alcoholism.		-	-
42.	Ct. G.	38 yrs.	1st 20 yrs. ago. Various attacks.	Wass. + Syphilis, pain in spine.	6/12/15	-	-
	"			Syphilis.	7/27	-	-
43.	P. M.	46 yrs.		"		-	-
44.	D. Dr.	44 yrs.	Old history, no discharge.	" (Wass. ±)		-	++
45.	S. S.	38 yrs.		"		-	-
46.	H. R.	42 yrs.	Denied. Smears.—	Stricture from injury.	6/12. 7/29 6/18	-	-
	"					-	-
47.	H. S.	50 yrs.		Syphilis, joint pains,		-	-
	"			" " " "	7/12	-	-
48.	C. E. M.	45 yrs.		" " " "		-	-
49.	E. A. E.	?		"		-	-
50.	R.	?	Only Gc., 1901. Clin. cure.	"		-	-

DISEASES OTHER THAN GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	DISEASE.	DATE.	P.	W.
51.	S. Mrs.	?		Syphilis ?		-	-
52.	T. Dr. J. S.	45 yrs.		"		-	-
53.	R. Mrs.	?		Came for Wass.-		-	-
54.	R. G. L.	29 yrs.		Syphilis.		-	-
55.	J. K. G.	34 yrs.	Denied. Chr. Disch.	Syphilis.		-	-
56.	N. H. W.	40 yrs.		"		-	-
57.	M. M.	34 yrs.		"		-	-
58.	A. S.	40 yrs.	Denied. Chr. Disch. No. Gc. ever seen.	Strictures.		-	-
59.	E. J. B.	38 yrs.	1901, 1903, 1906. stricture, clin. cure.	Syphilis.		-	-
60.	V. V.	35 yrs.	1900, 1906. Lues, 1906.	Tabes.		-	-
61.	M.M.			Came for Wass.-		-	-
62.	D. M. R. H.			Syphilis.		-	-
63.	B. "			"		-	-
64.	L. L.	32 yrs.		" ?		-	-
65.	C. A. M.			"		-	-
66.	A. C. N.	27 yrs.		Tbc. kidney.		-	-
67.	E. S.	27 yrs.	3 or 4 attacks, last 1912. Clin. cure.	Syphilis.		-	-
68.	F. Dr.	40 yrs.		"		-	-
69.	H. Mrs.		Denied.	Syphilis. Wass.+		-	-
70.	C. M.	37 yrs.		"		-	-
71.	C. S. J.	42 yrs.		Congenital "		-	-
72.	E. B.	34 yrs.	Only 1902. Clin. cure.	Hydrocele.		-	-
73.	T. H.		Denied.	Came for Wass.-		-	++
74.	J. S.	61 yrs.	"	Papilloma of cheek, probably on luetic base.		-	++
				Wass.+ 7/19		-	++
	"			7/30		-	-
75.	G. R.			Syphilis.		-	-
76.	E. M.	22 yrs.		Varicocele.		-	-
77.	L. Miss			Tumor of breast.		-	-
78.	B. H.	30 yrs.		Syphilis.		-	-
79.	A. E.	29 yrs.		"		-	-
80.	P.	?		Came for exam.		-	-
81.	H. L.	36 yrs.	1898, 1909. Clin. cure.	Impotence.		-	-
82.	H. K.	25 yrs.	Denied. Intermit. Disch.	Stricture.		-	-
83.	W. Mrs.		" " "	Syphilis (Wass.-).		-	-
84.	A. G.		Only 1914. Clin. cure.	"		-	-
85.	L. K.	30 yrs.	Only 1912. Clin. cure.	"		-	-
86.	G. B.	28 yrs.		"		-	-
87.	J. H.	24 yrs.		"		-	-
88.	Hosp. case.		Doubtful, supposed -	?	5/25	+	+
89.	A. F.	58 yrs.	No. Gc. history.	Tumor of Bladder.	5/28	-	-
90.	R. Z.	40 yrs.	"	Came for examination.		-	-
					5/10		
91.	H. M. S.	?	"	Bladder operation.		-	-
92.	J. C. C.	47 yrs.	Gc. 1895.	Post-urethritis.		-	+
				Smears-; bladder tumor.			
93.	Mrs. M.	?	No. Gc. history.	Fear after exposure.		-	-
				Smears.-		-	-

DISEASES OTHER THAN GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	DISEASE.	DATE.	P.	W.
94.	R. C.		No. Gc. history.	Alcoholism.		-	-
95.	Dr. M.		"	Came for examination.		-	-
				Lues.			
96.	M. R. H.		"	"		-	-
97.	C. Mrs.	30 yrs.	"	Acne rosaceae.		-	-
98.	A. D.		Obscure.	Rheumatism.	4/22	++	++
99.	A. D.	30 yrs.		Clear urine,	7/26	-	-
				shreds.-			
100.	W. H. R.	43 yrs.	1890 (lues, 1895)	Bright's disease.		-	-
101.	J. W. F.	41 yrs.	No. Gc. history.	Syphilis.		-	-
102.	Dr. T.	40 yrs.	Uncertain.	Came for exam. and		-	-
				Wass.			
103.	V. L. W.	43 yrs.	?	Syphilis.	- 8/5	-	-
104.	R. B.	46 yrs.	?	Hernia.	- 8/5	-	-
105.	S.	41 yrs.	?	Burn.	- "	-	-
106.	J. F.		?	Syphilis.	- "	-	-
107.	W. J.		?	"	- "	-	-
108.	P. D.	33 yrs.	?	"	- "	-	-
109.	J. K.	40 yrs.	?	Impotence.	- "	-	-
110.	W. H. R.	42 yrs.	?	Dyspepsia	- "	-	-

A review of Table I shows that the commercial antigen gave positive reactions in 5.4 per cent of unsuspected cases, and the W. antigen in 13.6 per cent. A majority of these positive cases gave histories of gonorrhea, and in the others it was impossible wholly to exclude a past infection. An illustration is found in Case 42. A barber denied gonorrhea at the time the test was made and later admitted various attacks, stating that his present condition gave him no trouble. Six weeks later the gonococcus test had become negative.

There is no relation between the gonococcic and the syphilitic antigens. In the table are 57 cases of syphilis. A majority gave positive Wassermann reactions, and negative gonococcus reactions. In 7 cases the W. antigen gave positive reactions, the P., 4. In these the Wassermann reaction was positive in 4, doubtful in 2, and negative in 1.

TABLE II.
ACUTE GONORRHEA.
(To the Fifth Week.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	GC. PRESENT.	DATE.	P.	W.
1.	J. L. P.	40	1895, April, 1915.	+	5/29/15	+	++
2.	W. P. G.	30	1910, 5/15/15.	+	5/28	-	-
3.	M. C. C.	36	1900, 1909. 5/7/15.	+	5/12	-	++
4.	H. B.	23	1914. 5/7/15.	+	5/7	-	-
	"	"	"	+	5/13	+	++
5.	A. M.	19	4/10/15.	+	5/10	+	++
	"			-	7/20	+	++
6.	H. B. S.	30	4/16/15. No Gc. after 4/19.	-	5/1	-	+
7.	M. W.	32	1908, 1913. 4/15/15.	+	4/26	-	++
	"			+	5/11	-	++
	"			-	6/2	±	±

ACUTE GONORRHEA. (Continued.)
(To the Fifth Week.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	GC. PRESENT.	DATE.	P.	W.
8.	Van D.	20	4/10/15. Marked cystitis.	+	4/26	++	++
9.	J. R.	69	4/15/15. Marked edema.	+	4/21	-	++
10.	P. B.	18	1914. 4/28/15.	+	4/28	-	++
11.	S. A. H.	27	1913. 5/5/15.	+	5/8	+	++
12.	St. C.	22	3 weeks' duration.	+		-	+
13.	L. K.	20	" "	+		-	+
14.	J. L.	20	2 " "	+		-	-
15.	A. F.	21	3 " "	+		-	-
16.	Case 2.		4 " "	+		-	++
17.	H. V. D.	24	1909 (lues, 1912) 5/4/15.	+	5/10	-	-
	"		" " "	?	6/4	++	++
18.	S. G. C.	35	1901, 1903, 1910. 6/5/15.	+	6/8	-	-
	"			+	6/20	-	-
	"			?	7/23	-	-
19.	L. C.		1906, 1909, 1911, 1914, 2 weeks' duration.	+		±	++
20.	T. J. B.	35	1910, 1914. 6/9/15.	+	6/15	-	-
	"			+	7/15	+	
	"			+	7/19	-	-
21.	A. C.	28	1911, 1912. 7/9/15.	+	7/16	-	-
22.	H. ?		1910. 7/15/15.	+	7/17	-	-
	"			+	7/26	+	++
23.	R. B.	40	7/20/15. (Lues. 4 yrs.)	+	"	±	++
24.	H. A.	25	1911. 4/26/15.	+	4/26	-	+
25.	A. G.	40	5/3/15. Vaccine.	+	5/5	-	±
26.	H. B.	45	? 1908. 7/25/15. Disch.	+	7/27	-	-
	"		Rheumatism,	+	8/13	±	
27.	G. C.	25	1913, 1914, 1915. 7/22/15. Disch.		8/13	-	-
28.	A. C.	28	1908, 1909, 1910, 1914. 7/25/15.		7/27	-	±
			Disch. vaccine.				
29.	C. B.	34	1913, 1914, to 5/11/15; 7/30/15.		7/30	-	-
			Disch. vaccine.				
30.	J. L. D.	22	8/1/15. " "		8/5	±	
31.	M.	"	1911. 7/25/15. " "		8/5	-	
32.	A. B. H. E. P.		? Epidid.	-	8/6	+	++
33.	" 9.		? " "	-	"	-	-
34.	" 10.		Three weeks. " "		"	-	±
35.	Sku.	27	1912, 1913. 8/9/15. Disch.	-	8/10	-	-
			Vaccine.				
36.	A. B. H.	23	Two weeks. Epidid.		8/13	-	++
			Very acute.				

The sera of the acute cases reacted with the commercial antigen 14 times, or 40 per cent. Of this number, only 2 cases were ++. The earliest reaction appeared on the fourth day, and three cases reacted within the first week. These figures show a higher percentage of positive reactions usually obtained with watery antigens within the first month of the disease. The W. antigen gave positive reactions in 26 cases, or 72 per cent. Of these, 18 were ++. Three occurred on the second day of the infection, and 8 within the first week.

Early reactions did not appear to have any relation to previous attacks of gonorrhea, as they occurred in primary infections, and were delayed in some cases that acknowledged many previous attacks. We have the impression that

those cases showing the greatest amounts of local reaction to the infection give the earliest positive reactions. The test was repeated in some cases after the expiration of the fourth week, but the results are included in this table.

TABLE III.
SUBACUTE CASES OF GONORRHEA.

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	GC. PRESENT.	DATE.	P.	W.
1.	Dr. O., 1.	26	6 weeks' duration. Epididymitis	+		-	+
2.	" 2.	28	5 weeks' duration. Epididymitis.	+		-	-
3.	A. B. H. 4.		Doubtful. Epididymitis.	-		-	-
4.	M. E. C.	20	2/1/15.	+	5/22	-	+
5.	Eal.	21	1913. 2/1/15.	+	5/12	-	-
6.	Temp.	20	1914. 2/1/15.	+	5/8	-	++
7.	H. H. H.	22	3/15/15? Rheum., five days later. Eye? No disch.	-	4/24	-	-
	"			-	5/5	-	-
	"			-	5/14	-	-
8.	C. H. McC.	25	3/4/15.	-	5/5	-	+
9.	A. L. M.	21	1913. 3/4/15. No. Gc. after 3/29.	-	4/3	-	-
10.	F. S. M.	30	2/20/15. Double epidid.	-	4/29	-	-
				-	5/6	-	-
11.	A. J. F.	28	3/5/15. Lame heel.	-	2/29	-	++
12.	B. L.	21	7 weeks' duration.	+	6/12	+	++
	"			?	7/19	+	+
13.	Dr. P.	30	1914. 6/7/15.	+	7/24	++	++
14.	F. A. E.	26	6/1/15.	+	7/15	++	++
15.	K. H.	23	1910, 1912. 3/1/15.	+	"	+	+
16.	A. P.	25	1911. 5/10 prost.	+	7/24	-	+
17.	Miss C.	20	? Bartholinitis, cervicitis.	+		-	+
18.	A. B.	26	5/15/15. Disch.	+	5/7	-	-
19.	Case B.		4/1/15. Clin. cure.	-	7/27	-	-
20.	G. C. R.		6/18/15. Disch, slight.	-	8/6	-	-

Of the 20 cases tested in the subacute stage, 4 responded positively to the commercial antigen, 11 to the fatty antigen, or 23 per cent and 64 per cent respectively. We observed in the cases noted in this and the following tables a considerable variability in the occurrence of positive reactions, a point of importance in connection with the low percentage. It has been observed by others,¹ and repeatedly noted in our own work, that alternating positive and negative reactions occur during the progress of an attack of gonorrhea, which appear to indicate phases of the infection. The precise significance of the fluctuations is not understood, but they appear to occur oftenest during the period designated as subacute. It is, therefore, not infrequent that a considerable series of cases will give negative reactions at one time, and positive reactions at another. It has seemed to us that the negative reactions were associated with lessened or absent discharge, and disappearance of gonococci.

Case 7 illustrates the value of repeatedly negative reactions. Early in the illness a slight discharge was noted, but no gonococci were ever found. The

¹Irons and Nickels: Jour. Infect. Dis., April, 1915.

case was pronounced to be gonorrheal rheumatism and iritis, but the serum reactions cast doubt on the diagnosis. The patient recovered promptly under salicylates.

TABLE IV.
CHRONIC GONORRHEA.
(Four Months to One Year.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	CONDITION.	GC.	PRESENT.	DATE.	P.	W.
1.	D. A.	25	12/1/14.	Stricture.		-	5/28	+	+
2.	K. H.	23	1910, 1912.	Ac. prostatitis.		+	5/26	-	-
	"		3/1/15.			+	7/19	+	+
3.	E. H. S.	37	1900. 12/26/14.	Discharge.		-	5/25	-	-
4.	A. B. H. 6.	?	Indefinite.	Smear.		?	"	-	-
5.	J. K.	?	8 months.	Strict.		-	5/7	-	-
6.	L. A. N.	34	12/14/14.	Disch.		+	5/12	-	-
7.	A. G. S.	23	Oct., 1914.	Stricture.		+	5/12	±	+
			Recur., 2/1/15.						
8.	A. K. F.	40	Dec., 1914.	Disch. (Lues)		+	5/6	-	±
9.	J. R.	?	1/25/15.	" "		+	5/6	+	++
10.	J. B.	?	Indefinite.	Prostate abscess.		Rectal ulcer.	4/29	-	-
11.	A. T.	21	6/1/1914. Recur., Dec., 1914.	Stricture.		-	5/8	-	++
12.	Case 1.	?	4 months.	Disch.		+	3/1	-	++
13.	Case 3.		4 months. -	"		?	"	-	++
14.	J. B. R.	22	Only 10/1/14.	" A. M.		+	6/4	-	-
	"			"		-	6/4	-	+
	"			"		-	7/19	-	+
	"			"		-	7/26	-	-
15.	L. A. P.	25	4 previous attacks, 2/1/15.	" A. M.		-	6/5	-	+
16.	H., Miss.	?	Indefinite.	Cystoscopy.		Ureteral Catheters.	6/5	+	++
	"						7/31	-	-
17.	X. M.	30	5 months healed epidid. No disch.			-	7/29	-	-
18.	L. V.	28	Only 7/1/14. No discharge since 4/1/15.			-	7/1	-	-
19.	A. U.	25	Months.	Threads only.		-	7/1	-	-
20.	O.	30	Indefinite, chronic.	Disch.		+	6/5	-	++
21.	A. B. H. 423-7.		" "	No disch.		-	"	-	-
22.	R. O.	30	" Gc. found once.	" "		-	5/13	-	-
23.	A. W.	?	Two months.	Disch.		+	5/13	++	++
24.	G.	"	" chronic.	No disch.		-	4/26	-	-
25.	W.	"	" "	" "		-	"	-	-
26.	A. M.	"	Five months, chronic.	Disch.		-	7/26	-	-
27.	C. H.	"	Six "	" A. M.		-	"	-	-
28.	O.	31	11/1/14. Rheum., 12/14.	No disch.		-	"	-	-
29.	W.	30	Doubtful.	" "		-	"	-	-
				Threads.					
30.	McN.			Disch.		+	"	-	+
31.	L. C. A.	26	1912. 1/1/15.	Strict. A. M. disch.		-	8/12	-	-

CHRONIC GONORRHEA (Continued.)
(Four Months to One Year.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
32.	E. W. L.	24	1910. 3/1/15.	Epidid. Clin. cure. Furnucle.	-	8/9	-	++
33.	Shel.	"	1914. et seq.	A. M. disch.	-	"	-	
34.	Kr.	30	Months.	"	-	8/13	-	+
35.	Hil.		1/1/14 et seq.	"	-	"	-	+

Thirty-five cases examined within the time limits of the table, but only 11 of which showed definite presence of gonococci, gave positive reactions with the commercial antigen in 6 or 17 per cent, and with the W. antigen in 17 or 48 per cent. It will be apparent that if the cases in this division had been limited to such as showed signs of remaining infection, in other words, omitting those free from discharge or other symptoms, the percentage of positive reactions would have been much higher. Number 14 well illustrates a periodical fluctuation in the course of a chronic case.

TABLE V.
LONG-STANDING AND REPEATED ATTACKS OF GONORRHEA.

NO.	NAME.	AGE.	HISTORY.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
1.	Dr. J.	44	Only 23 yrs. ago.	Sac. Iliac pain. Disch. slight.	-	5/15	++	++
2.	E. O.	28	1909, 1912, 1913, 1914, 1915.	Chronic epid. No disch.	-	"	-	-
	"			After exercise, disch.	-	7/12	+	+
3.	M. A.	40	1902, 1914. Rheum.	Well.		5/15	-	-
4.	A. B. H. 7		Indefinite.	4th epididyl.	-	5/25	-	+
	" (Gitas)				+		+	++
5.	" 8		Old and indef- inite-luetic.	No disch.	?	5/24	-	-
6.	J. S.		" " "	Disch.	?	5/24	+	+
7.	B.		1913.	Chr. post.	-	7/27	-	-
8.	M. A. R. C.		Old and indef- inite-luetic.	Disch.	?	"	++	±
9.	D. R.		" " "	"	?	"	++	++
10.	J. J. L.	49	Continuous for 20 yrs.	"	+	4/15	-	++
	"		After vaccine,	No. disch.	-	5/13	-	-
	"		" "	" "	-	5/20	-	-
11.	B. T. H.	51	4th attack in 14 yrs.	Strict. Disch.	?	5/25	++	++
12.	H. A. L.	29	1913, 1914. 4/21/15.	Disch., A. M.	-	5/20	-	++
13.	H. B. L.	36	1905, 1912, 1915.	"	-	5/20	-	++
14.	J. C.	?	1905, 1906.	Disch., A. M.	-	5/10	-	+
15.	P. H.	?	1903, 1909, 1914.	" "	-	5/14	-	+
16.	J. G. O.	?	1900 only.	(Lucs) Disch. A. M.	-	"	-	+
17.	L. K.	32	Only 1905.	" "	-	"	-	+
18.	G. D.	43	1909.	Strict. Disch. Epid.	-	4/1	-	-

LONG-STANDING AND REPEATED ATTACKS OF GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
19.	T. R.	65	1870.	Strict., 1885. Disch. A. M. Cloudy urine.	-	5/12	-	+
20.	W. J. S.	45	1895. 1904. Recur- rences.	Disch.	-	4/22	++	++
	"			" epididy.		5/10	-	++
	"			No disch.	-	7/15	-	-
	"				-	8/10	+	+
21.	J. C.	31	3 attacks.	Strict. pros- tatic. Disch.	?	5/8	-	+
22.	H. B.	28	1903, 1909.	" "	-	5/7	-	-
23.	A. L.	25	1911 intermit- tent.	" Disch.	-	5/6	-	+
24.	A. T.	39	4 attacks in 8 years.	"	-	5/6	-	-
25.	F. P. W.	44	6 to 8 attacks.	Strict. multiple. Disch.	-	5/6	+	++
26.	H. C.	32	1903, 1911.	Infiltrated urethra. Disch.	-	"	+	+
27.	H. I. A.	?	Old history.	No signs.		"	-	-
28.	H. D.	44	1892, 1897. Smeared 4/3/15.	After vaccine.	-	5/5	-	-
29.	F. P.	30	1911, 1913, 1915.	Strict.	-	"	-	-
	"			"	-	5/20	-	-
30.	P. R.	40	1904, 1909, 1914.	Strict. 1914, No signs.	-	5/5	-	-
31.	J. L.	44	Intermittent for 24 yrs.	Disch.	-	5/25	+	++
	"			"	-	5/20	++	++
32.	A. P.	30	2/1/13.	No signs, vague pains.	-	4/21	-	-
33.	C. L.	30	Rheum. 2½ yrs. ago.	Constant disch.	?	4/21	+	++
	"			" "	?	"	-	++
34.	F. A.	40	1901. Long use of sounds.	No disch.	-	"	-	-
35.	A. D.	35	1902, 1903, 1914.	Disch.	+	4/28	-	-
	"			No disch., urine clear.	-	6/8	-	-
	"			After epidid. clin. cure.	-	8/4	-	++
36.	H. C. C.	33	3 attacks to 1913.	Iritis, 2/26/14. Strict., 12/31/14. (P +). No Gc. ever found.		4/26	-	-
37.	C. W.	24	1908, 1914, 2/12/15.	Constant disch., prostatitis.	+	4/26	-	++
38.	G. K.	?	Old history strict.	No signs, Wishes to marry.	-	"	-	-
39.	A. H.	25	1911	"	-	"	-	-
40.	O. A. P.	30	1907, 1912. (Lues, 1910)	Prostatitis. Disch.	?	"	+	+
	"				-	7/12	-	-
41.	Z. C.	40	1901, 1905.	Intermit. disch.	-	5/10	-	-
42.	Case 4.	?	4 years' duration.	" "	+	3/1	++	++
43.	" 6.	?	Long recurring.	" "	+	"	+	+
44.	J. D. H.	28	1906 (psoriasis, 1911).	Disch. 10 days, prostatitis.	?	6/3	-	++

LONG-STANDING AND REPEATED ATTACKS OF GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
45.	A. R. F.	43	Only 1901.	Disch. A. M. Pain in back.	-	6/4	-	++
46.	A. H.	34	Only 1901.	Disch. A. M. Wishes to marry.	-	6/4	-	+
47.	A. S.	27	Only 1912. (Lues, 1908)	Marriage? No disch. Specks. Pain in shoulder.	-	7/29 6/8	-	± ++
48.	J. S.	44	1896, 1902.	Scrotal dermatitis.	-	6/4	-	-
49.	D. M. S.	30	Only 1911.	No signs. Wants children.	-	"	-	-
50.	B. H. N.	32	1912, 1914.	Intermit. disch. present.	-	6/9	-	+
51.	J. J. A.	39	1901, 1903, 1906.	No disch. Clinical cure.	-	7/15 6/9	-	-
52.	F. H. S.	30	1911. Seborrhea.	Wishes to marry. No signs.	-	6/12	-	-
53.	L. M. B.	59	Many attacks, 25 years ago.	Glycosuria. Prost. Disch.	-	"	-	++
54.	I. L.	50	1895. Many attacks (lues).	Disch.	-	6/15	++	++
55.	E. W.	56	Denies Gc. Has urethritis	" ? Prostatitis. Discharge.	-	7/21 6/12	-	-
56.	A. W.	38	4 or 5 attacks, last 1910.	No disch. Disch.	-	7/19 6/16	-	-
57.	G. F. D.	33	1903.	No signs sterile. ?	-	7/19 6/16	-	-
58.	Mrs. Z.	?	Gc. and lues, 1912.	No signs, sterile, ?	-	6/16	-	-
59.	H. F.	26	Test + 1912 lues.	Joint pains. No discharge.	-	"	-	-
60.	H. J.	26	1905, 1907, 1911, 1914.	No discharge. Pains in joints.	-	"	-	+
61.	J. N. W.	33	1900, 1904, 1909.	Recur. Vesiculitis. Stricture. Discharge.	?	6/12	-	++
62.	E. K.	28	1906, 1908, 1910, 1914.	Prostatitis. Discharge.	-		-	-
63.	H. L.	35	1903, 1914.	Intermit. disch. A. M.	-	7/15	-	+
64.	F. D. N.	51	1895, 1911. Irregular disch.	No disch.	-	"	-	-
65.	L. P.	36	1904, 1914.	Continuous disch.	-	7/17	-	-
66.	K. M.	32	1910.	No signs. Marriage?	-	7/15	-	-
67.	H. L.	40	1910.	Disch.	+	"	-	-
68.	A. R. W.	28	1909.	Pain in joints and kidney. No discharge.	-	7/19	-	-
69.	B. S.	37	1912 (Test+)	Slight disch.	-	7/24	-	-
70.	Dr. D. S.	?	Only 1904. Comp. cured. 1907.	Perineal pain. Disch pus. Diph.	-	"	-	-
71.	B. W.	48	2 attacks, last 20 years ago.	Enl. prost. Disch.	-	7/26	-	-
72.	J. L.	40	1893, 1900.	Impotence. Threads. Occasional disch.	-	7/26	-	-
73.	M. J. K.		1912, Comp.	Drinking. Disch. A.	-	"	-	-

LONG-STANDING AND REPEATED ATTACKS OF GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
74.	L. C.	50	1906.	Disch. 4 weeks ago. No disch.	-	5/10	-	-
75.	F. S.	58	1910.	Prostatitis. Discharge. Staphylococci.	-	4/29	-	-
76.	E. K.	26	1910. Recurrences.	No disch. Vesiculitis?	-	7/29	-	-
77.	W. Y. D.	30	1912.	Stricture, Specks, Smears. Retention.	-	6/12	-	-
	"				-	7/29	-	-
78.	E. S.		Indefinite.	Constant discharge.	+	8/5	-	++
79.	A. P.		1910 et seq.	Epidid. Orchitis. Disch.	-	8/13	+	++
80.	J. J. D.		1897.	Epididy. Vasitis. Sterile.	-	8/9	-	-

Eighty sera from cases of long-standing gonorrhea with repeated attacks gave 19 positive reactions with the commercial antigen, or 24 per cent, and 40 positive reactions with the W. antigen, or 50 per cent. Of these patients, 12 were clinically cured, 52 showed a discharge, and in only 10 were gonococci to be found. It is in this class that complement fixation is of the highest value. The percentage of positive reactions would be much higher, 37 per cent with the commercial antigen and 80 per cent with the fatty antigen, if the cases clinically cured and those free from discharge had been eliminated.

TABLE VI.
GONORRHEA IN CHILDREN (FEMALE).
(Unsatisfactory Histories.)

NO.	NAME.	AGE.	DURATION.	GC. PRESENT.	REMARKS.	DATE.	P.	W.
1.	S.	5	8 months.	+			-	++
2.	W.	3½	1 year.	-			-	-
3.	R.	8	"	-			-	-
4.	R.	3	3 months.	+			-	++
	"		4 "	?			-	++
5.	K.	8 mos.	3 months.	+			-	++
	"		4 "	+			-	++
6.	G.	5	4 months.	+			-	++
	"		5 months.	+			-	++
7.	H.	13 mos.	6 weeks.	+			++	++
	"		10 "	+			+	++
8.	P.	1 year.	3 months.	+			-	++
9.	F.	7 years.	1 year.	-	Diphtheroids. Gc. never found.		-	-
	"		13 months.	-	" "		-	-
10.	E.	10 mos.	2 months.	-	" "		-	-
	"		3 months.	-	" "		-	-
					(Faucial Diphtheria).			
11.	D.	2 years.	1 month.	-			-	-
	"		2 months.	-			-	-
12.	G.	15 mos.	8 months.	+			-	+
	"		9 months.	-			-	±

GONORRHEA IN CHILDREN (FEMALE.) (Continued.)
(Unsatisfactory Histories.)

NO.	NAME.	AGE.	DURATION.	GC. PRESENT.	REMARKS.	DATE.	P.	W.
13.	E. F.	7 years.	Years.	-	Gc. never found.		-	-
14.	R.	10 years.	?	+		5/4/15	++	++
	"			+		5/19	-	++
	"			-		5/28	-	++
	"			-	Vaccine.	6/30	-	-
15.	F. M.	5 years.	Months.	-			-	-
16.	I. C.	4 years.	"	-			-	-
17.	E. O. N. N.	8 years.	"	+			-	++
18.	D. P.	12	Long-standing.	+			-	+
			?	+			-	-
	El	"	1 month.	+			-	+
20.	F. Mas.	14	?	-			-	-
21.	L. C.	10	Months.	+			-	-
22.	T. S.	12	"	+			-	-
23.	M. W.	6	"	+			++	++
	"		1 month.	+			-	++
24.	A. T.	9	Months.	+			-	-
25.	S.	12 years.	Long-standing.	+		5/29	-	++
	"		vaginitis.			6/2	-	++
26.	P. M.	5	Long-standing.	+		5/6	-	++
			vaginitis.					
27.	C. B.	12	5 weeks ?	+		"	-	++
			vaginitis					
28.	N. B.	8	4 weeks	+		"	-	++
			vaginitis					
29.	M.	4	?	-		"	-	-
30.	O. F.	2	1 yr. Vaccine.	+		4/24	-	+
	"		Vaccine.	-		5/7	-	-
31.	S. I.	5	Doubtful.	-		4/29	-	-
32.	B. F.	8	Doubtful.	-		"	-	-
			Long-standing.			"	-	+
33.	R. C.	6	Vaginitis.	+				

Thirty-three female children, a majority with unsatisfactory histories, showed positive reactions to the commercial antigen in only 3 instances, less than 10 per cent, and to the W. antigen in 18, 54 per cent. We have been unable to account for the very low percentage in the former, unless the explanation may be found in the antigen. The W. antigen was made from various strains of gonococcus, 7 of which were isolated by one of us from cases of specific vaginitis in girl children, whereas we understand that few, if any, of the strains used in making the commercial antigen were obtained from such source.¹

Table VII shows the number of positive reactions obtained with both antigens in cases where gonococci were demonstrable. There is also shown the number of positive reactions where gonococci were not found. Certain apparent discrepancies in totals are explained by the fact that in some instances where the test was repeated on the same serum the gonococcus was present on one occasion and absent on another.

¹The vaginitis strains referred to have only recently been presented to the laboratory of Parke, Davis and Company.

TABLE VII.

	GC. PRESENT.	P.	W.	GC. ABSENT.	P.	W.
Acute	32 cases	12	24	7 cases	1	6
Subacute	13 cases	4	9	9 cases	1	3
Chronic	11 cases	4	8	22 cases	2	8
Long-standing	11 cases	4	8	60 cases	13	30
Children	20 cases	4	18	16 cases	0	3

Analysis of the above shows, on the whole, what appears to be a lower percentage of positive reactions with the commercial antigen than has been obtained and reported by other workers. There are several factors to be considered in this connection. By far the greater number of cases, in fact nearly all, except the children, who were hospital cases were private patients, and received the best of care. The complicated cases, usually neglected, and ultimately found in dispensaries and hospitals, are few. Among the latter the proportion of positive reactions is much higher. Again, tabulation of cases of the class dealt with according to the age and duration of the infection, some of the cases appearing clinically cured, must necessarily yield a lower proportion of positive reactions than a series in which all but the doubtful cases have been excluded. A third factor is the variability or fluctuation of reactions that occur during the course of an infection. It has been observed in this work, and by other writers, that alternation of positive and negative reactions occur frequently during the continuance of gonorrhea. The reason for this is not explained, although it is unquestionable that exercise, alcohol and sexual excitement have decided influence. The administration of antigen in form of ordinary vaccine has no influence whatever. With these facts in mind, it is not difficult to understand how one series of cases may show more positive reactions than another.

There is no question but that a single negative reaction in a suspected case has no value, whereas repeated negative reactions in the absence of clinical signs of gonorrhea are of great value. We strongly urge repeated tests in doubtful cases giving negative reactions. On the other hand, a single positive reaction, even in the absence of clinical signs, is of the greatest importance and indicates sensitization by gonococci. Our experience has shown that stimulation in such cases, with sounds and silver, frequently is followed by the appearance of cocci in the exudate.

The effect of epididymitis upon the complement fixation has not been marked in this series or in our previous work, and we believe this phase of the disease to have no influence in producing positive reactions, but rather the contrary. We have observed repeatedly negative reactions throughout the course of epididymitis, and have observed positive reactions disappear with an oncoming attack. The severity of the complication has appeared to be associated with negative reactions, mild cases giving a much higher proportion of positives. Theoretically, one would expect such relationship in view of the well-known clinical phenomenon of disappearance of exudate and cocci from the urethra during an inflammation of the epididymis, a few of the severest cases of which unquestionably induce cures of the urethral infection.

Comparison of the two antigens shows the superiority of the Warden antigen over the best of the watery antigens. Where the latter gave positive re-

actions, the former invariably gave positives, and, as a rule, to a greater degree, save in one instance only. With the latter also there is much less fluctuation. The positive reactions appeared earlier, persisted longer, and occurred in a larger number of doubtful cases. We believe this antigen can be still further improved. The data in this work appear to us to warrant the following conclusions:

1. An alcoholic solution of the fats of the gonococcus serves as antigen in the gonococcus complement fixation test, and is superior for the purpose to the watery antigen of commerce.

2. We concur in the opinion that positive reactions are always of value. Repeated negative reactions in the absence of clinical signs are of great value. A single negative reaction has no significance whatever.

3. While the sera of normal persons have been thus far wholly negative, and while it is admitted that positive reactions are largely confined to cases where the gonococcus is, or has recently been, present, nevertheless, the evidence, as a whole, leads us to believe that a positive reaction indicates the presence in the serum of some substance which reacts with the antigen (antibody?) to produce fixation of complement, and not necessarily the presence of a focus of gonococcus in the body.

We wish to thank Dr. E. W. White and Mr. Richard Zickman for much assistance in the preparation of this paper.

FETAL ERYTHROBLASTOSIS: FETAL ERYTHROBLASTOMATOSIS *

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

CONGENITAL general edema is a subject which has been engaging more and more attention. About it a very considerable literature has been developing, not because the cases which exhibit the condition are very frequent, for they are not, but because of the opportunities for theoretic perambulations. Ballantyne, Chiari says, was able to find but 70 cases in the literature between 1614 and 1898. Recently, especially since the awakening of interest by the communications of Schridde, the reports are becoming more frequent.

In its characteristic form, congenital general edema is evident at birth, and appears to have developed during the period of the fetus in the uterus. Because of the swelling of the tissue, delivery is difficult, and after the expulsion or forcible removal of the infant, the cord and placenta are, like the child itself, found to be waterlogged.

Concerning the causes of the condition, something is known of some cases; nothing, of others. They are grouped as follows:

1. Hydrops ascites congenitus caused by disturbances in the portal circulation; fetal peritonitis; absence of the ductus venosus Arantii; and in certain anomalies of the intestinal tract.

*From the Mary M. Emery Laboratory of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.

2. In a certain group of cases there are anomalies of the heart, of the vessels, or of the uropoietic system of the fetus.

3. In a certain group of cases the edema of the fetus seems to be associated with maternal disease—chiefly nephritis.

4. In another group none of the above mentioned factors are present, and in this it has been supposed that some metabolic anomaly of the fetus develops which finds expression in abnormal blood production and edema.

It is this last group which is most interesting, for in it belong the cases of what has been called fetal erythroblastosis (Raubmann). This very peculiar and almost generally unrecognized complex is characterized, as Schridde said, by the following features:

1. The fetuses (premature as a rule) show universal edema in the form of anasarca, and hydrops of the body cavities.

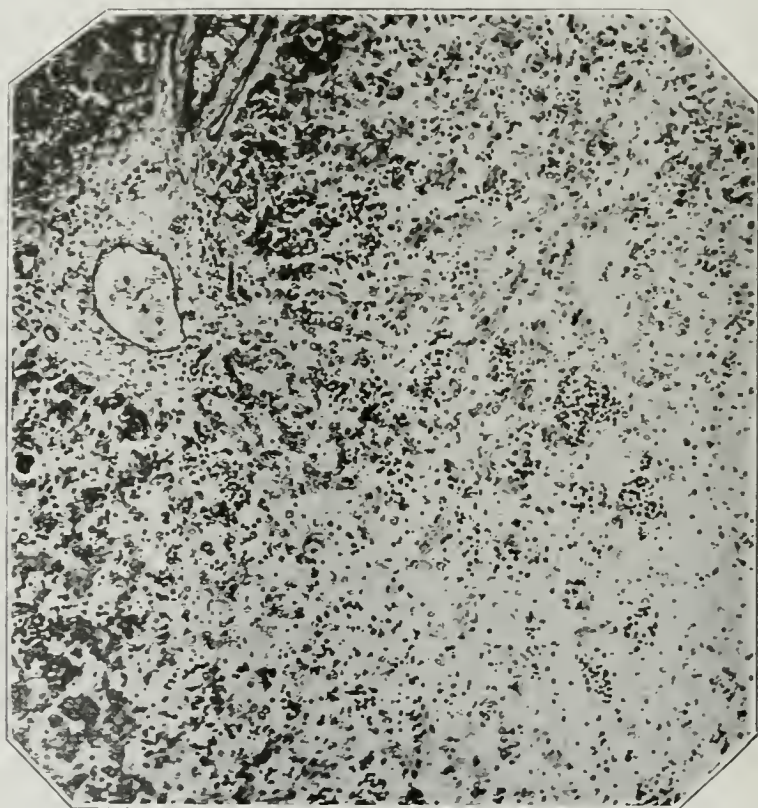


Fig. 1.—A low power photomicrograph of a liver section to show the islands of (chiefly) erythroblasts and the atrophy of the hepatic parenchyma.

2. Most of them show also edema of the placenta and cord.

3. The liver and spleen are markedly enlarged.

4. Microscopic examination of the liver and spleen show unusually large numbers of erythroblasts with large numbers of other myeloid cells, inside and outside the vessels. Also, the liver cells are atrophic, and in the spleen the follicles are absent. In other organs, especially in the kidneys and lymph nodes extramedullary erythroblastic nodules appear, and the blood shows enormous numbers of erythroblasts overshadowing all other elements. Mitotic and rhectic figures appear, and the liver cells and pulp cells of the spleen are colored with iron-containing pigment.

These characters were shown in their entirety by a case sent me by Dr. H. L. Woodward, which, however, was more than usually interesting in that

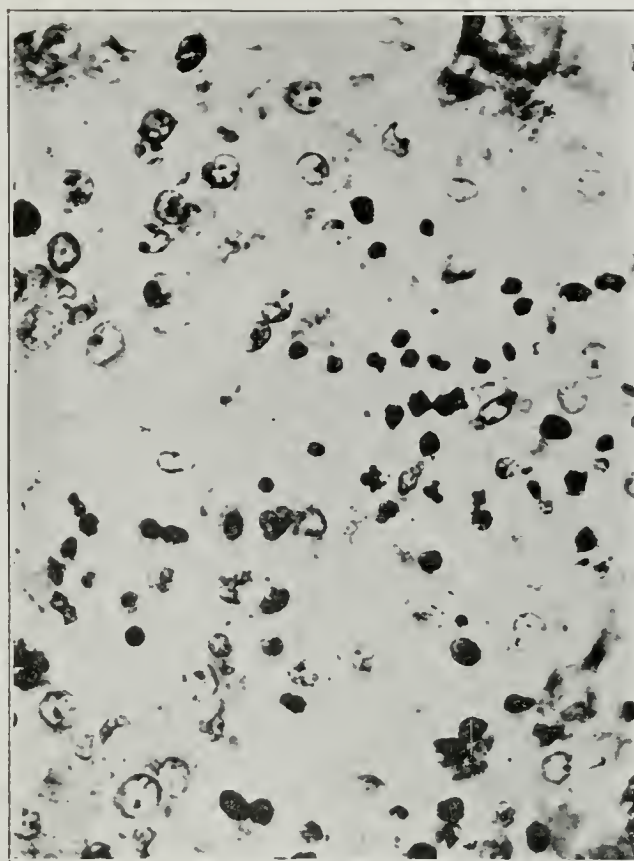


Fig. 2.—An area in a section from the liver showing the edematous, and atrophic liver cells, and also numbers of erythroblasts, some of them showing mitosis.

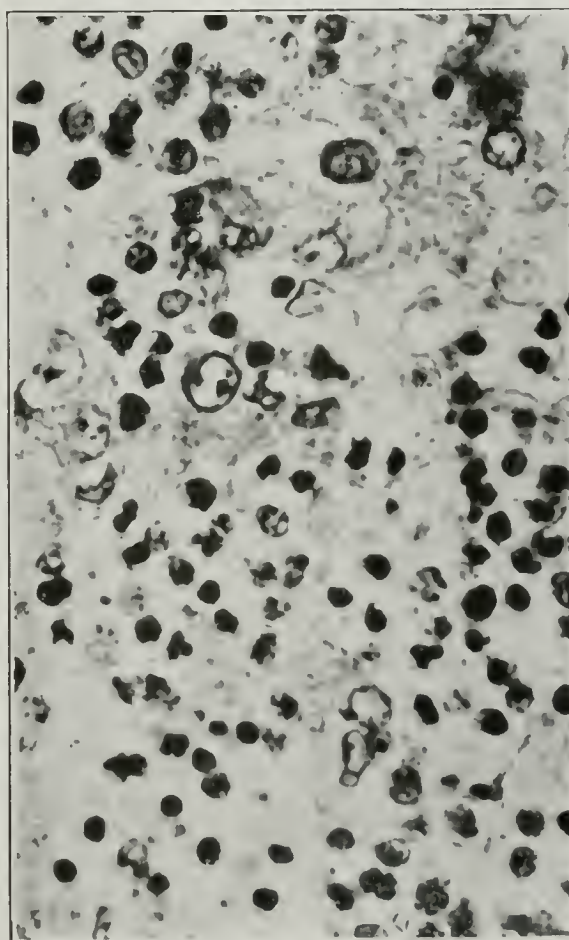


Fig. 3.—Same as Fig. 2, but somewhat greater magnification. The parenchymatous changes are well shown.

the anasaruous fetus was one of a pair of twins. The other twin was apparently normal. Delivery was not forcible, but the delay caused by the delivery of the anasaruous infant led to the death of the healthy one. Both were premature.

Schridde believed that this fetal disease was the result of a severe fetal anemia which was associated with a high grade of reparatory erythropoiesis. The anemia, it was assumed, was of toxic origin. Schridde did not believe it

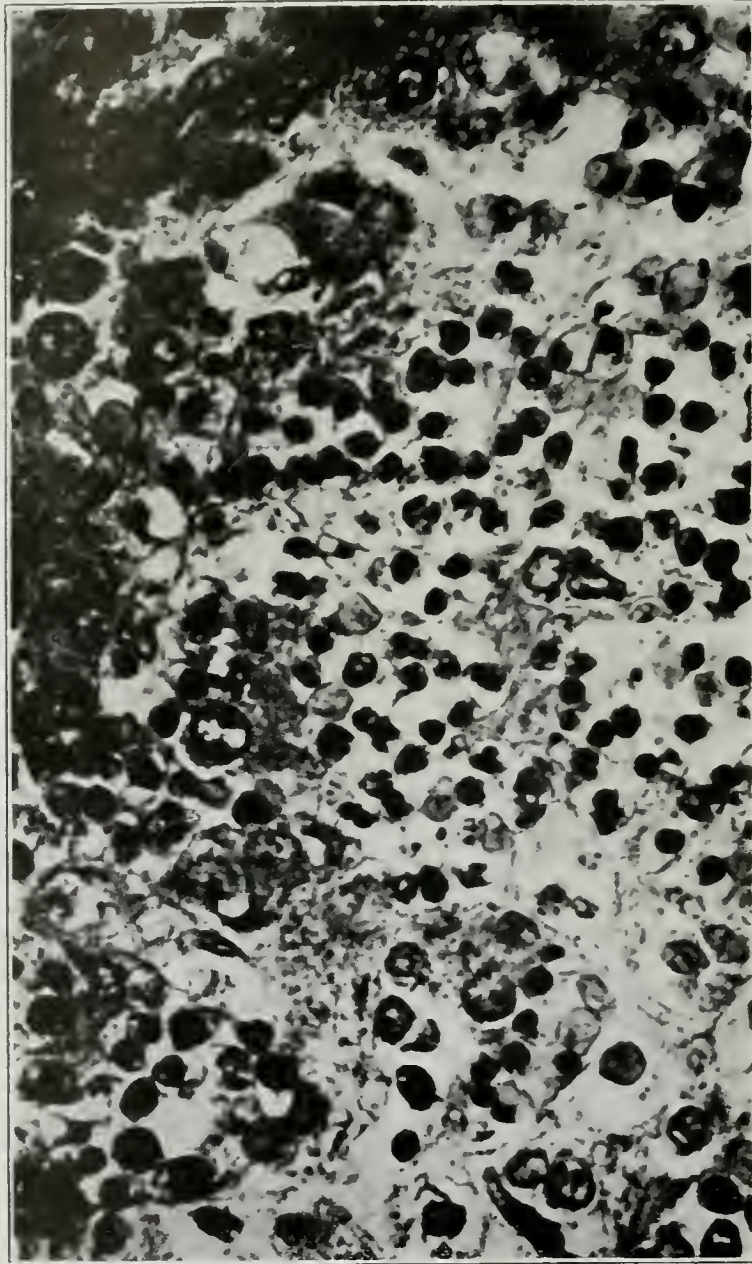


Fig. 4.—Same as Fig. 3, but with still greater magnification.

to be luetic. Fischer called attention to the similarity of the picture to that of *anemia pseudoleukaemia infantum* and suggested that this kakophoniously named disease might be an exaggerated form, or a further development, of fetal anemia in which the criteria might be similar to, though less marked than those of the erythroblastosis type. One of Schridde's cases was derived from a nephritic mother but in this instance he believed that the fetal condition was coincidental and not the result of the maternal condition. This is interesting because there is the tendency to lay these cases at the door of maternal disorders

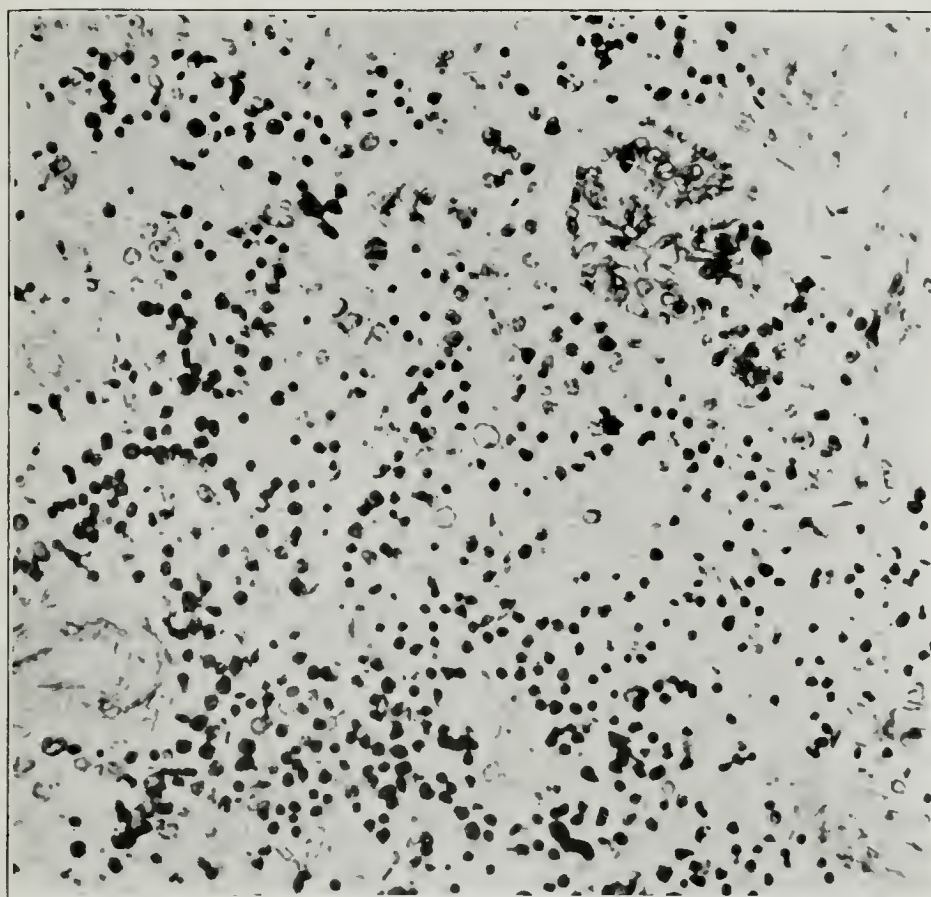


Fig. 5.—A low power photomicrograph of an area in the kidney showing the massing of erythroblasts.



Fig. 6.—Same as Fig. 5, but with greater magnification.

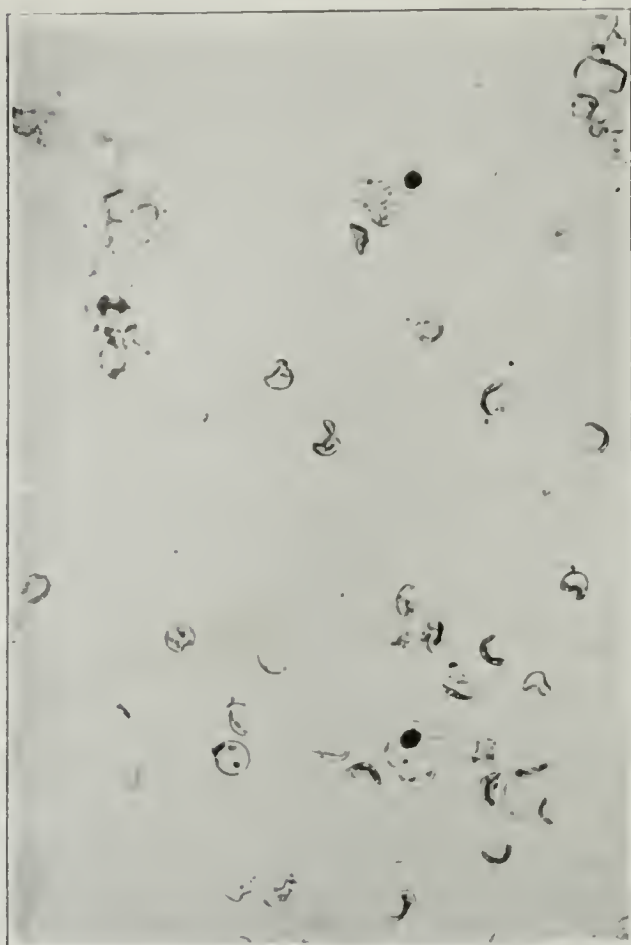


Fig. 7.—Nucleated red cells in the umbilical vein.



Fig. 8.—Same as Fig. 7, but with greater magnification. One erythroblast shows evidence of division.

(Fischer, Wolff). It is generally supposed that the disease is of toxic origin and that the toxic materials, while causing destruction of erythrocytes, at the same time stimulate the blood forming tissues. The origin of the poisons is not known. Certainly they do not come from the maternal blood—or almost certainly. In the case here reported, one twin was healthy.

Schridde and Chiari both incline to the opinion that the condition rests upon some fundamental disturbance of the metabolism of the fetus, though what the cause is they do not know. The question remains whether there is a chronic irritation of the erythroblastic tissues, or whether the primary thing is a destruction of circulating red cells with reparatory overproduction, or both.

It would appear that the anasarca is the expression of the anemia which



Fig. 9.—A placental villus with nucleated red cells in its vessel.

in turn is due to disturbed production and perhaps also to increased destruction, of erythrocytes. The pigmentation of the organs speaks for such destruction. As is very well known, edema is a constant occurrence in all grave anemias, because either there are not enough red cells to carry oxygen to the tissues, or because the hemoglobin content of the cells is not sufficient to attend to the oxygen needs. In either case, the oxidations of the tissues are necessarily slowed and metabolites which normally would be broken down and carried away remain in the tissues, and, because these metabolites are in the nature of acids, they lead to water absorption by the tissues. The anasarca is therefore the expression of a severe grade of anemia. When anatomic defects occur in the body, anasarca also occurs because the blood, capable though it

may be, is not properly oxygenated for obvious reasons. In typical erythroblastosis, there are no anatomic anomalies.

Why the anemia occurs is a more difficult question. There seem to be two possibilities. There may be some chemical irritation of the hematopoietic apparatus which, while it causes overproduction of erythroblasts, also causes increased destruction of erythrocytes. The evidence is not good for establishing a relationship between maternal conditions and the fetal state. In the case here noted, one twin was healthy; the other, erythroblastic. Nucleated reds appeared in the fetal circulation but not in the maternal. The condition may also, so writers say, be the expression of a general metabolic anomaly. This, of course, is begging the question. Nevertheless, it may be suggested that there is something of an analogy between this condition and leukemia. Leukemia is often considered a form of neoplastic overgrowth, in which the tissue concerned in the production of white blood cells, is affected. It seems that if that conception has any force, and we believe it has, then erythroblastosis may be looked upon also as belonging to the group of tumors, and in this case it is the erythroblastic tissue which is involved. If this should appear to be true, then the term fetal erythroblastomatosis would be significant.

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LABORATORY METHODS

A New Operating Table and Head Holder for Experimental Purposes*

BY D. E. JACKSON, M.D., ST. LOUIS, MO.

THIS table is made almost entirely of ordinary gas pipe and gas pipe fittings. It is intended that, in so far as possible, all the parts shall be of regular standard make and size and therefore easily obtainable. The table can be folded up completely as shown in Fig. 2. The table complete, including head holder and manometer stand weighs approximately fifty pounds. The frame, legs and braces (*c*, *d*) are made of half-inch pipe and fittings. The table is 35 inches high.

The wooden top (*a*) is 3 feet 8 inches long by 12 inches wide and 1 inch thick. The metal frame beneath this is 3 feet 5.5 inches long by 13.5 inches wide as measured from center to center of the pipes. The rear legs (*e*, *e*) pass upward and screw into side-elbows which are connected by close nipples with tees which are bored out large enough to turn loosely on the pipe which forms the rear end of the framework. The other openings of the side-elbows are connected by a short piece of pipe which holds the upper ends of the legs firmly in place. When the brace (*c*) is screwed out the rear legs are then thus free to rotate backwards and be folded completely back up over the top of the table. Two small notches are cut in the rear end of the top of the table to admit the revolving tees as the legs are folded back over the table top. Similarly the front legs (*f*, *f*) pass upward beneath the table but about 1.5 inches in front of the end of the wooden top. The legs here screw into side-elbows. These side-elbows are connected by a short piece of pipe which holds the upper ends of the legs firmly in position, while the other opening of each of the side-elbows (toward the table top) is connected (by a close nipple) to a tee which is bored out to turn easily around the pipe which forms the front end of the frame. When the brace (*d*) is screwed out the front legs are thus left free to fold backward up under the bottom of the table.

The pipe *l*, which is covered by a cap at the top, passes down and screws into a side-tee, the side opening of which is turned toward the table top and is connected by a close nipple with another side-tee, the side opening of which is turned down and carries a set screw to fasten these two side-tees and the pipe *l* in any position desired; for the straight opening of each of the two side-tees is bored out to slip easily over the two pipes which are located at the front end of the table, viz., the front end of the frame of the table and the pipe which connects the two side-elbows into which the upper end of the front legs are screwed. These two pipes are parallel and about 2.25 inches apart (from center to center) and the sliding of the two side-tees from side to side on these pipes permits the

*From the Department of Pharmacology of Washington University Medical School, St. Louis, Mo.

pipe *l*, and hence the head holder *k*, to be moved from side to side to any desired position.

The braces (*c* and *d*) are screwed into tees, the threads of one end of each brace being left-handed. The pieces *d*, *c* serve as handles for screwing in or out these braces. At the upper end the braces are screwed into the side open-

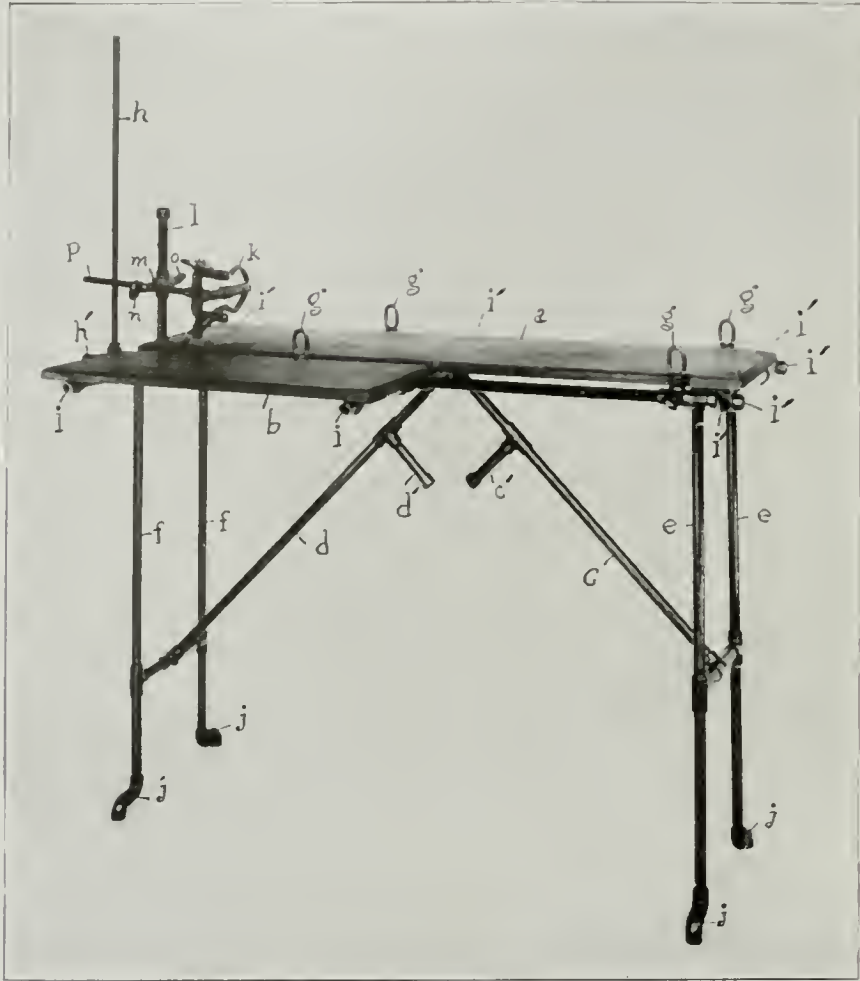


Fig. 1.—For description see text.

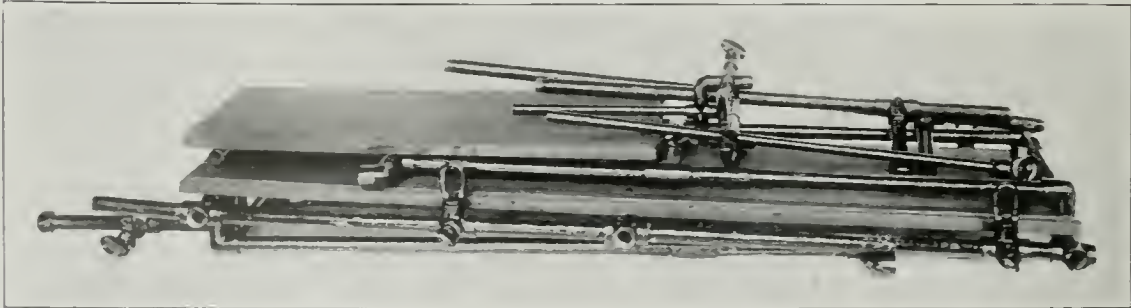


Fig. 2.—For description see text.

ings of a side-tee which is placed in the middle of the central cross pipe of the frame of the table.

The shelf (*b*), is movable and can be easily shifted to any one of four positions or left off entirely. Or a long shelf, the full length of the table, may be placed on one or both sides, or short shelves may be placed at one or both ends of the table. These various changes are made possible by screwing short

pieces of pipe (*i, i*) into the side openings in the frame of the table. There are ten of these openings, a part of which are indicated by the letter *i'*. The shelf (*b*) is held on to the short pipes (*i, i*) by small flat metal clamps such as are used to attach gas piping to walls, etc. The shelf can be quickly slipped off the pipes until the dissection is complete and then replaced to hold apparatus, etc.

At the floor each leg carries two elbows (*j, j, j, j*), joined together by a close nipple. This gives the table a wider base and prevents overturning. Castors may be attached if desired.

The loops (*g, g, g, g*) are used for the attachment of cords which hold the animal, and are half-inch size chandelier loops screwed into the upper openings of side-tees. The other openings (lateral) of the side-tees carry set screws. The straight opening of each of the side-tees is bored out to slide or turn easily on the side pipes of the frame. Thus by means of the set screws these loops may be quickly fastened at any desired position to fit various sized animals.

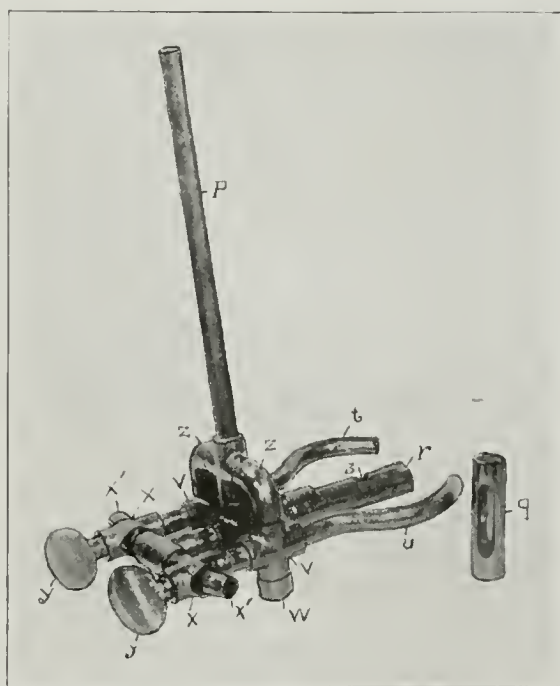


Fig. 3.—For description see text.

The pipe *h* (which holds the manometer, tambours, etc.) is quarter-inch sized piping and is 24 inches high. At the bottom it screws into the upper opening of a side-tee while the other (lateral) opening of the side-tee carries a set screw to fasten the side-tee (and hence the pipe *h*) in any desired position on the short pipe *h'*, which is screwed into one of the side openings (*i'*, not indicated at this place in the picture) of the frame. The straight opening of the side-tee is bored out so that it will slide or turn easily on the pipe *h'*.

At *m, n, o* in Fig. 1 is shown the method of attachment of the head holder (*k*). By means of the adjustments here indicated the head holder can be adjusted in any direction, laterally, up and down, back and forth, and it can be rotated entirely around or placed at any angle as is often desirable for operations on various parts of the head.

The head holder (*k*) (Fig. 3) can be entirely detached from the table and fastened on the animal out in the room. The head holder may also be

used on any ordinary table or operating board by simply attaching the pipe *l*, by means of a flange to the top of the table or board. The head holder is made of quarter-inch tubing and fittings in all parts except the piece *r* (Fig. 3) which is of half-inch piping. Near the center of this piece *r* is a hole one-half inch in diameter shown at *s*. This hole extends entirely through the piece *r*, and is for the purpose of admitting a stomach tube if one cares to administer a drug in this manner.

The piece *q*, is a wrench used to turn the various set screws about the table. It is a piece of half-inch pipe 3.5 inches long with a slot cut in one side.

The pieces *t* and *u* (Fig. 3) are 4.5 inches long and the curved part which passes around the animal's jaws is 3 inches long. Each of these curves should be wrapped with several layers of gauze which is tied on tightly by twine or soft copper wire. The animal's canine teeth are passed in front of the piece *r*, and then the pieces *t* and *u* are quickly closed down tightly on the outside of the jaws. The set screws *y, y* are quickly tightened and the animal is held secure, thus avoiding largely the use of a muzzle. The crosses *v, v* are bored out to turn freely on a 2.25 inch nipple which screws into the elbow *z*, and is capped by the cap *w*. The cross *x* is bored and filed out to slide freely back and forth on the short curved pipe *x'*. It is intended that this head holder should be quickly and effectively fastened on to the animal and that the pipe *p* may be used as a handle to carry out any further manipulations of the animal.

Any ordinary plumbing or gas fitting establishment can readily and cheaply furnish all the metal parts of this table and head holder. The wood top and shelf (or shelves) can be obtained in most places without difficulty.

Some of the ideas incorporated in this table have been suggested by Mr. John A. Higgins.

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EDITORIALS

The Measurement of the Mass Movement of the Blood

THE object of the circulation is to supply the tissues with blood, and the adequacy of the supply will depend mainly on the difference of pressure between artery and vein and the calibre of the intervening vessels. It is impossible, from the blood pressure measurements alone, to form any accurate estimate of the rate at which blood is flowing through an organ or part (the so-called mass movement of the blood). It often happens, for example, that perfectly normal blood pressure values may be obtained from the vessels of a part (such as the hands or feet) the blood supply of which is nevertheless obviously either above or below the normal as indicated by the subjective sensations of heat or cold; thus, cold hands or feet may be complained of when blood pressure measurements in the artery and vein give normal values. This fact suggests a method for measuring the real blood supply, namely, to ascertain the amount of heat which the part gives off. Such a method has been worked out by G. N. Stewart, who has used it in the study of a series of clinical cases with results which cannot but be of importance and value in the clinic.

Indeed, Stewart has been able in many cases to detect changes in blood-flow where no such change was suspected, and by repeating the measurement from day to day, in cases where the blood-flow was found to be inadequate, he has been able to supply information without which it would have been im-

possible to decide on the correct line of treatment. We will go into some of these cases in greater detail later on, but meanwhile it may be of interest to point out *the general principles of the method*.

The apparatus consists essentially of a vessel containing a known quantity (3,000 c.c.) of water and a thermometer from which a change of temperature of a hundredth of a degree Centigrade can be read. In order to diminish as much as possible the loss of heat between the vessel and the outside air, the walls of the vessel are double, the space between being stuffed with broken cork. The top of the vessel is closed with a thick cork plate, having suitable openings in it for the hand or foot and for the thermometer and a stirrer (feather) with which to keep the water in constant motion. The apparatus is called a *calorimeter*.

After the hand or foot has been in the calorimeter, with the water a few degrees below that of the body, for a certain time, the temperature of the water will, of course, become raised and the degree to which this occurs, multiplied by the volume of the water in cubic centimeters, will give in calories the amount of heat dissipated. By the application of a very simple formula it is now an easy matter to calculate how much blood must have passed through the blood vessels of the part in order to give out the observed amount of heat; for, if *we divide the calories by the difference in temperature* between the inflowing and outflowing blood of the part, the result must indicate *the volume of blood*, in cubic centimeters, that has passed through it. Thus, if Q equal the amount of blood, H , the calories of heat given out to the calorimeter, T , the temperature of the arterial blood, and T' , the temperature of the venous blood, then we have the equation: $Q = \frac{H}{T - T'}$. The only other point in this equation that we need dwell on at present is the measurement of the temperature of inflowing and outflowing blood. It has been shown by Stewart that the temperature of the inflowing blood is the same as that of the mouth, or 0.5° C. below that of the rectum, and that the temperature of the outflowing blood equals the average temperature of the water in the calorimeter during the observation. To allow for the specific heat of blood, the result is multiplied by $\frac{10}{9}$.

Theoretically, then, the method is very simple, and there are no unusual technical difficulties in carrying it out. The only special precaution is that the air surrounding the calorimeter should be kept tolerably constant in temperature, so that we may be enabled to allow in our calculations for the loss of heat from the calorimeter itself, this value being obtained by observing the change of temperature in the calorimeter for a certain period of time after the hand has been removed from it. The observation should, therefore, be made in a small room with closed doors, or if it must be made in a ward, the space around the bed should be well surrounded with curtains.

The results are calculated on the basis of grammes of blood flowing through 100 c.c. of tissue in one minute. In a normal, healthy individual the average flow in the hand is from 12 to 13 gms. for the right hand, and about half a gramme less for the left. This difference between the two hands corresponds, of course, with their relative degree of development. The average foot-flow is much less, and varies according to whether the patient is sitting up or lying

down while the measurement is being made. In a normal individual, while lying down, it was 5.11 gms. in the right foot and 5.23 gms. in the left, per 100 c.c. of foot; but only 3.96 gms. for the right, and 4.1 gms. for the left foot, while sitting up. The results have been found to undergo only a slight variation from month to month in a given healthy individual, provided the air temperature during the different observations is the same. To obtain these constant results, however, it is very important to have the person long enough in the room before the observations are begun, especially if he be a dispensary patient and has been in the open air with bare hands. Approximately standard conditions of room temperature must become established if comparison of flow in the same patient at different times or of different patients is proposed. Finally, it should be remembered that, although the flow under standard conditions is very constant for each individual from time to time, yet it varies in different individuals, both with regard to the absolute amount of the flow and the ratio between the two hands or feet. When the total flow in the two hands is compared with that in the two feet, it is usually found that a ratio of about 3 to 1 is obtained.

Before proceeding with the results obtained in clinical cases, it may be of interest to consider some of the physiological causes for variations in blood flow. As above indicated, the most marked of these is probably *the temperature of the room*. *The temperature of the water in the calorimeter* has likewise a great influence, and for the comparison of different cases it is always important that the room and calorimeter temperatures be stated alongside the results. *Muscular contractions*, produced by moving the fingers in the calorimeter, were found to produce an enormous change in flow; thus, with calorimeter temperatures of 24.38° C. and 23.64° C. for the right and left hands respectively, and a room temperature of 21° C., the flow was 15.4 gms. per 100 c.c. per minute of the right hand while it was being made to contract in the calorimeter, and only 4.9 gms. for the left, which remained at rest. Since 4.9 gms. is a much smaller flow than was ever found in the hand of this person with both hands at rest, the increased flow in the contracting hand was evidently accompanied by a diminished flow in the hand that was at rest. *Moderate constriction of the arm* so as to obstruct the venous circulation caused a great diminution of flow, which however increased later because the blood began to pass the constriction, on account of the rise in venous pressure. By increasing the constriction of the arm up to and beyond the systolic pressure, as estimated by the clinical method, no blood-flow occurred, and as the pressure was gradually reduced below the systolic, the flow only became slightly increased for a considerable decrement of pressure. This result indicates that the pressure in the armlet must fall somewhat below the diastolic pressure before any marked increase in the flow through the hand occurs.

The most interesting physiological condition that causes changes in the blood flow is a *vascular reflex* set up by immersing the opposite hand or foot in hot or cold water. In the former case the blood-flow through the observed hand increases, while in the latter it decreases. This change may be of a temporary character, or it may persist throughout the whole period of immersion of the hand. Constriction elicited by cold seems to be more durable in the foot

than the hand, and this fact must be borne in mind when making foot measurements. The practical importance of having a reflex which we can observe quantitatively, is, of course, very great, for it permits us to study the effect of lesions affecting either the nerve or the nerve center concerned in vascular reflexes.

The reliability of the method was evidenced by the results obtained in *clinical cases in which obvious differences existed* in the blood-flow of the two hands or feet, such, for example, as in a case with a recent cicatrix of one arm, and in one in which one of the arm nerves had been severed. The most interesting point observed in these cases was that the vascular reflex did not behave in the normal fashion, being either greatly exaggerated or depressed. The vasomotor control of the new blood vessels of the cicatrix, or of the new nerve fibers in the regenerated nerve, was evidently very unstable. Several cases were observed where the venous return from the hand was interfered with by a tumor. Marked diminution of blood-flow was observed, which was not the case, however, in other cases where edema existed as a result of lymphatic obstruction. The value of the method in diagnosing the edema due to lymphatic or venous obstruction is thus illustrated. In a case of inflammatory redness and swelling of the hand as the result of an infection, a considerable increase of blood-flow was observed, but, still more interesting, a great decrease in the flow in the normal hand also occurred. The suggestion is made that this curtailment of flow in the normal hand occurs for the purpose of providing an increased flow in the infected area. In other words, in order to provide for an increased flow in the infected area, a vasoconstriction, possibly elicited reflexly through the pain nerves of the infected hand, is brought about elsewhere and particularly in the symmetrically placed part of the opposite side. Furthermore, it was observed, in this infected case, that the immersion of the opposite hand in cold water caused scarcely any reduction of the flow in the infected hand. The vasomotor impulses therefore for some reason had become blocked. In other cases of nonbacterial inflammation of the hand, as in gout, no sign of vasoconstriction block was obtained.

We will now pass in review certain groups of clinical cases in which a change in blood-flow might not be detected by the employment of ordinary clinical methods.

Anemia.—The blood-flow in the hand was found to be smaller than normal in cases of pernicious anemia, chlorosis and secondary anemia. The deficiency was less, however, in the chlorotic group than in the other cases of anemia. Sometimes the degree of deficiency was very marked; thus, in a case of marked secondary anemia, the flow was only 1.26 gms. per 100 c.c. of hand for the right, and 1.18 gms. for the left hand. Since it has been shown by Plesch that in anemia the minute volume of the heart is increased, it must follow that the vasoconstriction at the periphery will compel more blood to pass through the lungs; in other words, that it is a compensatory arrangement for increasing the flow through the lungs, so as to make up for the deficiency of blood.

Fever.—Since changes in the cutaneous circulation probably constitute the chief factor in the derangement of the temperature-regulating mechanism in

fever, it is evidently of great advantage to be able to measure such changes quantitatively. This has been done by Stewart in several cases of typhoid fever and in one case of pneumonia. The general result has been that the flow in the feet never exceeded the normal flow, and was usually much below it. This tendency to vasoconstriction seems to be carried into convalescence. For practical reasons the hand-flow has not been so extensively studied. This hyperexcitability of the vasoconstrictor mechanism at the periphery is most naturally interpreted as a defensive reaction of the organism by which an increased supply of blood is imported to those internal organs which bear the brunt of the infection. When we consider that in spite of this constriction at the periphery the blood pressure is low and the pulse dicrotic, we must conclude that there is considerable dilatation of other vascular parts, especially the splanchnic area. A very practical application of these facts presents itself in considering the rationale of the cold bath treatment for fever. If, for example, we conclude that the cutaneous constriction is in the interests of an increase in the blood-flow to the organ on which the stress of the infection falls, it will evidently be more rational to lower the temperature by methods which will not diminish, and may even increase, the cutaneous constriction than to do so by causing the vessels to dilate. In other words, the use of antipyretics seems to be contra-indicated, since they diminish the body temperature, causing vasodilatation at the periphery with a consequent withdrawal of blood from the seat of infection. Such drugs "diminish the temperature, it is true, but at the cost of defeating the beneficial redistribution of the blood, which it is the function of the peripheral vasoconstriction to ensure."

Cardio-vascular Diseases.—Taking all the observations that have so far been made on *cardiac cases*, certain general conclusions, which are only provisional in some instances, may be drawn. The hand-flow is far more apt to be markedly deficient in cases where there is evidence of serious impairment of the myocardium than in cases where gross valvular lesion exists while the heart action is strong and orderly. This indicates that it is more serious for the pumping force of the heart to be interfered with than for its valves to be leaky. No matter how tight the valves may be, the output will be small and irregular if the stroke of the pump is feeble. This general statement applies more particularly in the case of mitral than of aortic insufficiency. Even where there is considerable venous engorgement, the flow may be little diminished, provided the myocardium is not impaired, and a failure of the myocardium will immediately be indicated when a marked diminution in the hand-flow occurs with great engorgement of the veins. Stewart and R. W. Scott have recently reported observations on cases of auricular fibrillation treated with digitalis. In untreated cases the blood-flow was subnormal. In three patients the blood-flow was promptly and decidedly increased after the administration of digitalis, but in a fourth case, which had not been considered to respond well to this drug, the blood flow was somewhat increased when the drug was stopped after a long course of it. The general conclusion is that in cases of fibrillation, which are evidently benefited by digitalis, the blood-flow through the hands becomes coincidentally increased.

As would be expected, *arteriosclerosis* was found to be associated with a small blood-flow, and the vasomotor reflexes to be weaker than in normal per-

sons. In *thoracic (aortic) aneurysm* the flows were either of the normal order of magnitude or at least not so conspicuously insufficient as might be expected. Of course where the aneurysm was of such a size as to cause pressure on the subclavian artery or vein, a diminution in flow of the corresponding hand was observed, but otherwise aortic aneurysm in itself, although it may cause great changes in the character of the pulse beat, does not evidently affect the mass movement of the blood. In a case of aneurysm of the subclavian artery, more recently examined, the blood-flow was much greater in the corresponding hand (12.52 grams per 100 c.c. of hand per minute) than in the opposite hand (6.36 grams). Special interest attaches to the case because the amplitude of the pulse was very obviously *less* on the affected side than on the sound side, and, judging from this alone, misleading conclusions regarding the mass movement of the blood might have been drawn. The difference between the systolic and diastolic pressures (the pressure pulse) was found to be much less on the affected side (*S.* 110, *d.* 88 mm. Hg.) than on the normal (*S.* 130, *d.* 70 mm. Hg.). By clinical blood pressure measurements, therefore, false estimates of blood-flow are quite likely to be drawn.

The increased blood-flow was no doubt due partly to vasodilatation brought about by pressure of the aneurysm on the brachial plexus and partly to the lower resistance to the flow of blood into the dilated right subclavian. The pressure on the plexus interfered not only with the tonic vasoconstrictor impulses but also with those to the skeletal muscles, for there was marked loss of power as well as pain in the affected arm. Eleven days after ligation of the right innominate the flow in the corresponding hand had of course diminished (to 5.44 grams), but the point of particular interest is that the flow in the opposite hand had reciprocally increased. "The cutting off of the path through the innominate and right common carotid obviously permitted more blood to enter the alternative route of the left subclavian and left carotid."

The gradual development of a collateral circulation could be very accurately followed on the operated side; in 17 days after the operation it was 4.76 grams, in 24 days it was 4.86 (cool morning), and in 31 days, by which time the hand was being freely used, it was 8.55 grams. Even with this reestablishment of adequate flow no pulse was palpable on the right side.

Interesting observations were also made on cases of *Raynaud's disease*. As would be expected, the flow was small, the diminution being more or less proportional to the duration of the disease. The contralateral vasomotor reaction to cold was also peculiarly intense. In diabetic gangrene of the feet a very subnormal flow was also observed in both the hands and the feet. The vasomotor reflexes were also feeble.

Besides cardiovascular diseases inequality in flow in the two hands may be due to *nervous conditions*, and a recent paper deals in detail with the characteristics by which the exact cause of the inequality may be diagnosed. When due to mechanical causes, the inequality remains unchanged over short periods of time, whereas in nervous conditions the inequality changes from day to day. Of course, over longer periods the mechanical inequalities may also change, as, for example, in cases of thrombosis or embolism. Another important distinction is brought out by testing the general vasomotor reflexes by changing the temperature of the room and observing whether the ratio between the two hands

remains unchanged or becomes altered. If it becomes altered, the inequality must be due to nervous causes. As a practical method of testing these cases, Stewart suggests that the blood-flow measurement should be made at intervals of several hours apart on the same day with different room and calorimeter temperatures. As illustrating the importance of these facts, Stewart details the results obtained in a case of embolism and thrombosis involving the left arm and right leg. The ratio in the hands was 1:2.7. By repeated examination it was found that the ratio between the two feet became progressively larger, until at last it mounted to 1:9.28, thus indicating a tendency to gangrene in the foot with the curtailed flow. The interesting point in this case, however, was the fact that when the ratios of the sum of the foot and hand flows were compared over a period of four months, they were found to be practically constant, namely, 1:1.90. That is to say, even though one foot had become much less vascular than the other, the ratio of blood flowing to the hands and feet was maintained at its usual level, thus showing that the blocking of the vascular path to one leg was associated with a reciprocal dilatation of the path to the other leg, so that the normal partition of the blood between the legs and the rest of the body was scarcely disturbed. This indicates that the blood which normally finds its way through the two common iliacs seems eventually, when the main part of the path from the one common iliac is blocked, still to find its way through the one which remains pervious, room being made for the additional quantity of blood by vasodilatation. It is further suggested that this adjustment occurs in such cases so that there may be no interference with the elimination of heat by way of the extremities. A certain amount of cooling has to be effected through the extremities, and if the circulation through one of these should be curtailed, then that through the other must become reciprocally increased. This adjustment in chronic cases would not probably be evident when acute curtailment of the blood-supply to one limb is produced by ligation or amputation.

An interesting case given in detail is the one of *hematoma in the axilla*. The hematoma was due to a gunshot wound. The pulse was distinctly less on the injured side, and nutritional changes in the hand raised the question as to whether there existed a degeneration of branches of the brachial plexus, or whether the flow was merely mechanical, due to pressure of the hematoma on the artery or vein. By testing the vasomotor reflexes it was found that the cause was mechanical and not due to vasoconstriction. It was also important to decide in this case whether the curtailment of flow had become so marked as to indicate surgical interference, in order to prevent the onset of gangrene. It was found that there was enough blood in the hands (5.32 gm. per 100 c.c. per minute for the right, with a ratio of 1:2.77). Five days later the ratio was unchanged, so that the cause could scarcely have been vasomotor. One hundred and ten days later the right hand had risen to 9.3 gm. with a ratio of 1:1.24, indicating that great improvement in the blood-flow had taken place. Without the information furnished by the blood-flow measurements it would have been impossible to tell by palpation of the pulse whether in this case surgical interference was justified.

Diseases of the Nervous System.—The effect of *neuritis* on the flow varies with the duration of the case. In cases of early peripheral unilateral neuritis

there was an increase of flow bringing the ratio between the two hands to 1:1.34, with the greater flow on the diseased side. Though the vasoconstrictor reflexes were as great as normal, yet they were evanescent in character. These results are interpreted as being due to a partial paralysis of the vasoconstrictor fibers in the inflamed nerve. Similar vasoconstrictor paralysis, as we have seen, may also be caused by pressure of an aneurysm on the brachial plexus. In neuritis of long standing the flow was cut down, the ratio becoming 1:1.43, with the greater flow on the healthy side. The changes here are probably due to anatomical alterations in the lumen of the tube, perhaps a thickening of the intima, as had previously been suggested by Wingate Todd. In a case of *motor neurone disease* without any involvement of the sensory skin nerves the flow was normal, and the reflexes well-marked. The conclusion is that involvement of the muscle nerve does not interfere with blood-flow to anything like the same degree as does involvement of the skin nerve, the blood-flow through the skin being relatively more important than that through the muscles.

Hemiplegia.—A deficiency of blood-flow of the paralyzed side, varying however in degree, was usually observed, and the ratio may be 1:1.4 in favor of the healthy side. The vasomotor reflexes were found to be altered, the most usual change being that vasoconstriction was more easily produced than vasodilatation; indeed, in some cases there was observed an abnormal tendency to vasoconstriction.

Tabes dorsalis.—The flow was distinctly diminished, especially in the feet, although also in the hands, and the vasomotor reflexes were also quite feeble. Sometimes there was inequality in the flow of the two hands, which however need not necessarily indicate a unilateral lesion of the cord in the cervical region. In the discussion of these cases Stewart points to the value of the method in the detection of *malinering*. He cites the case of a patient in whom *malinering* was suspected, but in whom an examination of blood-flow revealed so small a foot-flow as to make it very probable that an actual pathological condition existed. Total absence of the vasomotor reflexes confirmed the diagnosis of *tabes dorsalis* as did also the further progress of the case.

—J. J. R. M.

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Concentration of Protective or Curative Bodies in Antisera

PASSIVE immunity, which may be of great importance in the prevention or treatment of disease, depends on the fact that the serum of an animal that has been actively immunized against a given microorganism contains certain principles which are able to protect another animal that is given an injection of this immune serum. This principle, first demonstrated by Richet and Héricourt in 1888, became at once of extreme importance following the experiments of von Behring and Kitasato who demonstrated that the serum of rabbits that had been immunized with diphtheria toxin contains a substance that neutralizes that toxin and thereby cures animals that are suffering from the effect of such toxin. In the sera of animals immunized against other bacteria or bacterial products, it has since been shown that various immune bodies other than antitoxins are present, such as lysins, agglutinins, tropins, and the like. These latter substances have an affinity for, as well as a harmful action against, the various microorganisms that have been employed to produce them, and in many instances give great therapeutic results. It is probable that failure to produce better results is due in a large part to the failure to understand the best method of producing these antisera, and particularly to the failure to appreciate the best method of administering them. This becomes evident when we consider the very striking results that have been obtained with at least one such serum—antimeningitis serum—described by Wassermann and particularly utilized by Flexner who obtained remarkable results following the intraspinal injection of the serum in epidemic meningitis.

One of the important lines of future progress in the employment of therapeutic immune sera lies in the methods of concentrating the active principles of the serum. The antitoxins, antimeningitis serum, and the like, are produced by immunizing large animals with the filtrates or cultures of the bacteria that they are to be used against, but it is often necessary to employ relatively large doses of the serum of such immunized animals to produce the desired results. Antisera of this type being derived usually from the horse are not entirely without untoward or even dangerous effects when injected into human beings, because they are foreign proteins and we know that the body reacts in certain definite ways to the injection of foreign proteins. Foreign proteins, when they are not split in the intestinal canal for further assimilation, but injected parenterally, may give rise to delayed symptoms, such as pains in the joints and an urticarial eruption. A second injection of such a foreign protein may give rise to an immediate reaction, which is known as anaphylactic shock, characterized by respiratory symptoms, and in some cases by sudden death.

The concentration and purification of these immune sera, then, would bring about two desirable results. It will first of all remove a large percentage of those proteins in the foreign serum which do not contain the antibodies, and secondly will concentrate those remaining proteins which are found to contain the active therapeutic principles that it is desired to utilize. The Gibson method of concentrating antitoxins is a chemical means of producing these desiderata. This method, which is currently employed for concentrating diphtheria antitoxin, consists in precipitating out the globules of antitoxic serum from the horse by means of one-half saturation with ammonium sulphate and further re-

moving the globulins that are not soluble in a saturated solution of sodium chloride which leaves behind the globulins that contain practically all the antitoxins. Antitoxins prepared in this manner may be greatly concentrated so that one cubic centimeter will contain many times the number of antitoxic units that are present in one cubic centimeter of the original serum, and the antitoxic globulins prepared in this way furthermore do not seem to contain the irritative substances of the horse serum which give rise to urticaria.

It was further shown, however, by Banzhaff and Gibson¹ that the globulins are increased during immunization of horses against diphtheria toxin and they have found that the globulins which contain the antitoxin rise from 60 to 90 per cent of the total globulins present. So in employing the concentrated serum, although one avoids the injection of the total amount of protein in the corresponding original serum, yet a relatively large amount of foreign globulin is introduced.

Another method of concentrating the antibodies of immune serum might be devised by using the specific precipitin reaction. This reaction, as is well known, depends on a peculiar property that an immune serum derived from immunizing animals with bacteria, or with soluble foreign protein substances, contains a body known as a precipitin which, when the serum is mixed with antigen, gives rise to turbidity, floccillation and the formation of a precipitate. This test is most frequently employed in medico-legal tests for human blood where it has proved most valuable. It was originally suggested by Kraus in a combination of bacterial extract and its corresponding immune serum. Considering the question of an immune serum derived from the horse, we may produce a precipitate either by adding an extract of the bacterial substance that had given rise to this serum to it, or by adding to such a serum the serum of another animal, let us say a rabbit, that had been immunized against horse serum. In the first of these cases the immune serum acts as a precipitin, namely, when the precipitinogen is added in the form of a bacterial extract; in the other case the horse serum acts as the precipitinogen. In both cases a precipitate is produced, but the amount of horse serum necessary to produce the maximum precipitate varies greatly in the two types of reaction. When the immune sera acts as a precipitin it may be added in excess, but when the immune sera is the precipitinogen it must be added in relatively dilute form to produce the maximum precipitate.

That reactions of this type may be of practical importance in concentrating the curative properties of immune sera was first shown by Dehne and Hamburger² who added rabbit antiserum to antitoxins from the horse and found that the antitoxin was brought down in the precipitate thus formed. These experiments were made with tetanus antitoxin and similar ones with diphtheria antitoxins by Atkinson and Benzhafl.³ Such results, while of great theoretical interest, are not of practical significance in concentrating antitoxins, owing to the fact that the antitoxins are brought down only when great excess of rabbit antihorse serum is added, which would preclude the practical use of this method for the concentration of large amounts of horse serum.

The reverse of this reaction has been shown to be possible in at least one combination, by the work of Gay and Chickering⁴ who found that those substances which protect animals against infection by means of pneumococcus pres-

ent in the sera of horses immunized against this organism may be concentrated in the precipitate produced by adding an extract of pneumococcus to this horse-antipneumococcus serum. In such a reaction, as will be seen, the component necessary in excess is the immune serum (precipitin) and for this reason large amounts of such immune serum can be completely precipitated by adding relatively small quantities of the extract of pneumococcus. These precipitates are found in the experiments of Gay and Chickering and later by Chickering,⁵ to contain practically all the protective antibodies of the original serum, and moreover to contain relatively small amounts of protein as compared with the original serum, often as little as 1/30 or 1/50 of the protein of the original serum in aliquot parts. It is evident that these protective bodies are simply adherent in some mechanical way to the precipitate for they can be leached out from the precipitate by dilute sodium carbonate at 42°. This relatively pure antibody solution of low protein content is now being tried in the place of the original serum in the treatment of some cases of pneumonia with apparent promise of as good results as those obtained with the original serum.

These observations on the concentration of antibodies in pneumococcus serum naturally led the author to further studies of other antibodies of various sorts. The first report on these studies will shortly appear in the *Journal of Immunology*, but it may be stated that they have, so far, been rather discouraging and it would seem that the case with pneumococcus serum is the exception rather than an example of a general law. It may well be, however, that the method employed with other types of antiserum has not been the one best adapted to produce the results desired. At least other attempts are indicated in this field which may lead not only to practical results but to results of great theoretical interest when one considers the possibility of studying antibodies in perhaps the purest form in which they have been obtained.

—F. P. G.

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Diphtheria Carriers

A RECENT investigation of diphtheria carriers in Detroit is reported by Goldberger, Williams and Hachtel, in Bulletin No. 101, of the Hygienic Laboratories, of the United States Public Health Service. The problem of diphtheria carriers has become one of considerable importance and has been given special prominence of recent years by the studies of von Scholly, Moss, and Nuttall and Graham Smith. The writers of the report mentioned above studied 4,093 people in the city of Detroit, and found that 0.928 per cent harbored bacil-

li identical morphologically with the Klebs-Loeffler bacillus. This figure is rather lower than those of some other investigators, but would indicate, as stated by the writers, that there were from 5,000 to 6,000 diphtheria carriers in the city of Detroit.

Of 19 cultures isolated from 19 of the carriers, only two were virulent, which would indicate that only 0.097 per cent of the people examined carried organisms capable of producing disease. An interesting further point is that the bacillus *Hoffmanii* was present in at least 41.9 per cent of over 2,000 individuals examined, and that the 49 cultures morphologically identified as bacillus *Hoffmanii* were avirulent. This would confirm the impression gained, we believe, by most experienced laboratory workers, that a true *Hoffmanii* can be distinguished with considerable certainty from a Klebs-Loeffler bacillus by morphological examination alone, and that its significance is probably that of a frequently present saprophyte of the throat and pharynx. The studies of Goldberger, Williams and Hachtel also indicate that in examining for diphtheria carriers, it is best not to confine oneself either to the nose or throat, but that cultures should be taken from both places in every case.

—H. Z.

Erratum

IN the January issue of the Journal in the article by Dr. Roger S. Morris on "Methods for the Determination of Glucose in the Blood" the first sentence on page 254—"For example, 21 c.c. of blood filtrate, representing 3 c.c. of blood, gave a titration of 5.5 c.c. $\frac{N}{10}$ permanganate," should read as follows: "For example, 21 c.c. of blood filtrate, representing 3 c.c. of blood, gave a titration of 5.5 c.c. $\frac{N}{50}$ permanganate."

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ORIGINAL ARTICLES

OBSERVATIONS ON TYPHOID FEVER IN DETROIT

BY HENRY F. VAUGHAN, EPIDEMIOLOGIST, AND W. FRANK WALKER, SANITARY ENGINEER,
DETROIT BOARD OF HEALTH.

THE City of Detroit located on the Detroit River just below Lake St. Clair covers an area of approximately 47 square miles. The United States Census estimate of population for 1915 was 554,717. There is reason to believe, however, that the true population is somewhat in excess of this figure. This is based on the fact that Detroit's growth for the last decennial period was not uniformly distributed; approximately 60 per cent of the increase occurring between the years of 1906 and 1910. This growth parallels the growth of the automobile industry in the city. Because of this irregular increase, the increment based upon the average increase for the last decade and used in making up the United States Census estimate is too low. A census taken by the Water Board, based upon the number of families multiplied by a factor, which has been found by comparison with the United States Census to be fairly reliable gives the population for 1915 as 673,498.

The average width of the river at Detroit is 2,200 feet, with an average depth of 37 feet. The flow of the river as estimated by the United States Engineers is 209,000 cubic feet per second. Gaugings show that approximately 65,000 cubic feet per second flow through the American channel. The river near its head receives the flow of Connors and Fox Creeks which furnish respectively 780 and 150 cubic feet per second at times of flood.

Practically the entire lake traffic passes through the Detroit River, the number of vessels being about 38,000 annually with a net tonnage of 63,000,000 of which about 3,000,000 have Detroit as their destination. Of the 15,000,000 people carried annually by passenger steamers on the Great Lakes, very nearly 10,000,000 are carried by boats operating out of Detroit. Of these 10,000,000 of people nearly 4,000,000 are carried on boats running to cities or resorts at some distance from Detroit either up or down the river. The remaining 6,000,000

are carried by the ferry boats plying between Detroit, Belle Isle and Windsor. Thus Detroit's position is unapproached by any other city on the Great Lakes.

The climate of Detroit is that of the lower Great Lakes Region with a mean

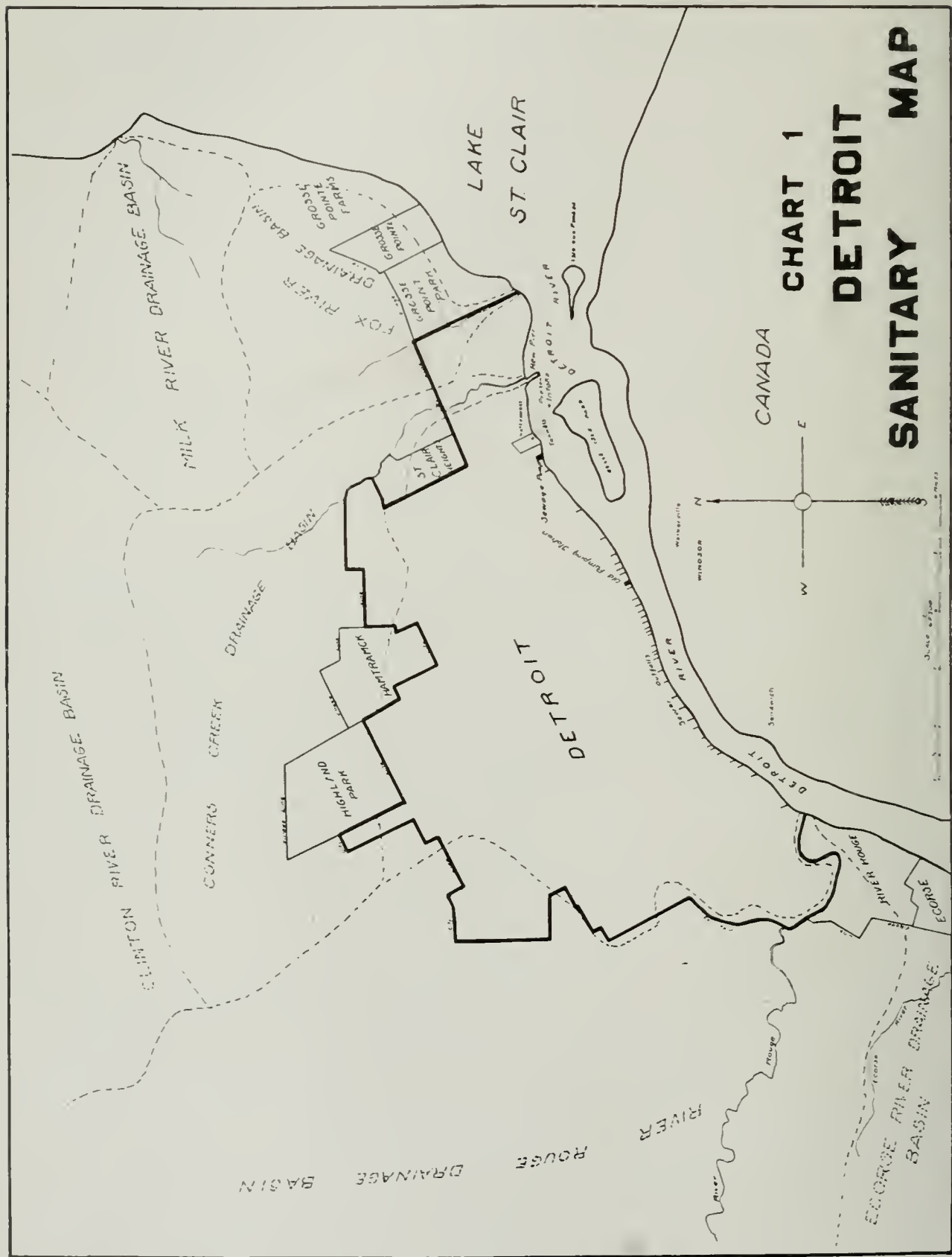


FIG. 1.

annual temperature of 48.2° F. with variations between 24.4° F. for February and 72.1° F. for July, the greatest daily variation being about 34° F. The mean annual precipitation is given as 32 inches, with a minimum monthly aver-

age of 2.09 inches for January and a maximum monthly average of 3.64 inches for June, the maximum for any one day being 4.57 inches. Wind velocities of 40 miles per hour are not uncommon, the average being about 13 with a maximum of 86. The general direction of the hard winds is from the West or Northwest.

GENERAL HEALTH CONDITIONS.

The following table gives, by years, the death rate per 100,000 in Detroit from typhoid fever, scarlet fever and diphtheria:

YEAR	TYPHOID FEVER	SCARLET FEVER	DIPHTHERIA
1900	28.4	6.3	29.1
1901	20.1	18.0	18.0
1902	23.5	24.2	44.4
1903	20.0	7.1	71.7
1904	17.6	11.3	44.4
1905	20.3	9.1	34.7
1906	20.7	28.1	22.6
1907	25.5	17.7	19.6
1908	19.6	17.7	16.1
1909	20.5	22.3	24.3
1910	23.0	16.2	33.8
1911	16.0	13.2	34.3
1912	17.5	11.8	37.3
1913	29.4	21.3	57.6
1914	13.8	9.5	29.6
1915	13.0	4.5	20.9

It will be seen that in 1914 there was a reduction from 1913 in diphtheria of 48 per cent, typhoid fever 53 per cent and scarlet fever 55 per cent. Likewise 1915 shows a marked reduction over the year immediately preceding. At this time the activities of the Health Department were materially extended and, it is believed, the efficiency greatly increased by the application of new methods. It is of interest to note at this point the relationship existing between the addition of hypochloride of lime to the water, the application of other preventative means for the control of typhoid fever, and the number of deaths by months. Referring to Fig. 16 it appears that the number of deaths from this disease continued to increase after the addition of the disinfectant to the water supply. We might possibly have had a much higher death rate had this form of treatment been omitted but still it seems reasonable to believe that, had the greater part of the typhoid fever at that time been due to the water supply, there would have appeared a reasonable reduction in the numbers of deaths during the months immediately following that date. The reduction of typhoid did not take place until 1914 during which year vigorous efforts were made to prevent contact infection, river water infection, boat infection, milk and food infection, etc. It was at this same time that the reduction became apparent in diphtheria and scarlet fever due to extensive preventive measures. The infant mortality rate was likewise reduced from 136.4 per 1,000 births in 1913 to 120.4 in 1914 and 104.6 in 1915.

A sanitary survey of the city made in 1913 disclosed some six thousand privy vaults within the city limits. The role that these insanitary fixtures are capable of playing in the spread of typhoid fever is well known. This has been

amply demonstrated by the board of medical officers in 1898 and likewise by the reduction, from over 100 deaths per 100,000 in 1910 to less than 30 in 1912, from typhoid fever in Jacksonville, Florida, through the passage and enforce-

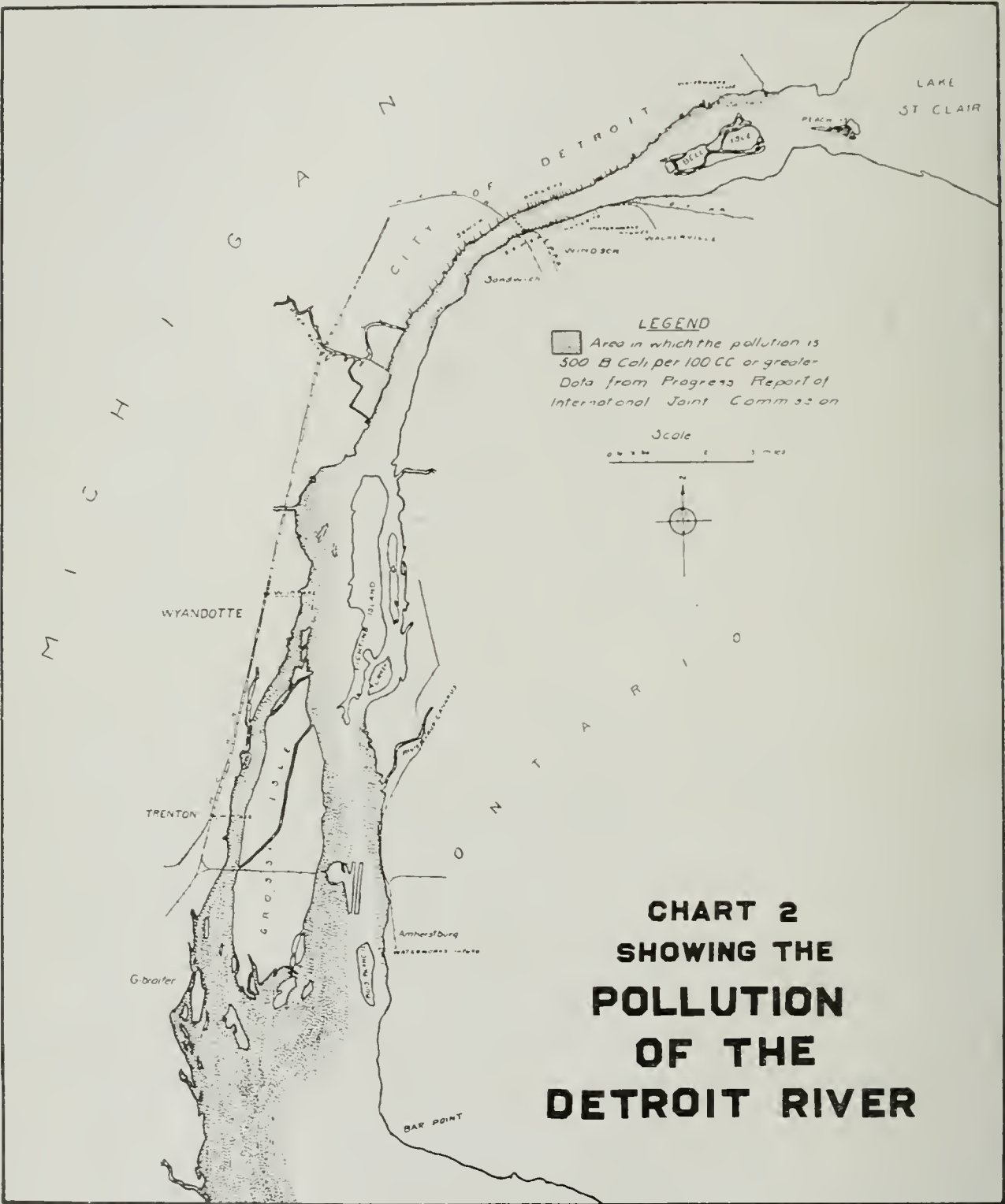


FIG. 2.

ment of a sanitary privy law. In Detroit, privy vaults have been removed and sanitary toilets installed as follows:

1911—1912	492
1912—1913	1,839
1913—1914	1,490
1914—1915	1,340

There are now probably less than 2,000 remaining in the city and these are being replaced as rapidly as time will permit.

During the past summer an extensive antily campaign was maintained under the jurisdiction of the Board of Health in which some four thousand boys participated. The city was divided into six hundred small districts and each boy was given a map of his district, literature and rules, and was made responsible for his block or group of blocks. This campaign aimed rather at the prevention of flies by destroying the breeding places than at the extermination of great numbers.

SOURCES OF INFECTION.

In order to obtain uniform and complete data with regard to each case of typhoid fever reported to the Health Department, the usual form of investigation card has been adopted. The following items are included: name, address, sex, color, nativity, occupation, visitors, visits made; date of first symptom, date of taking bed, date of doctor's first call, doctor, date reported; schools, number in family, adults, children, servants; previous typhoid histories and dates, personal contact, at home, at business, in cars; sanitation; milk; drinking water, present, 2 to 3 weeks before sick; ice; fresh vegetables, oysters and food, dealer's name; hospital; tests and results; termination; length of time in city; previous residence; boat and other excursions during the past month, date, locality, and drinking water; associates on excursions, their histories; bathing places and dates; visits to hospitals, etc.

The results have been found far more trustworthy when obtained by assigning one especially instructed investigator to the typhoid work than by relying upon the district nurse to obtain the information in the course of her other duties. This same nurse likewise instructs the members of the household regarding the care of the patient and makes the necessary demonstrations to assure the carrying out of all disinfections. Literature, amply illustrated, is provided describing the cause and methods of prevention.

Up until the spring of 1914 typhoid fever was not sufficiently reported in Detroit to justify any extensive epidemiological studies. At that time through the medium of the leading newspapers which printed appropriate charts and diagrams on their first pages and by court action, suitable publicity was given to the subject with the result that during the eighteen month period, July 1, 1914, to January 1, 1916, there have been 884 cases brought to the attention of the Health Department. The majority of these were reported by the attending physicians and a few were picked up by the investigating nurse. The accompanying table (Fig 3) gives the number of cases and deaths by months.

Dr. Wm. H. Price, Health Officer, in the January, 1916, bulletin states: "During 1915, 50 per cent of deaths reported as typhoid fever had not previously been reported as cases. Failure of these reports cripples the Board of Health both in taking of necessary measures for sanitary control and in providing accurate information for formulating a city policy relative to water supply and sewage disposal. The Board of Health has attempted through publicity, personal interviews and prosecutions to correct this failure of reports both regarding typhoid fever and other reportable diseases."

TYPHOID CASES AND DEATHS BY MONTHS.
DETROIT, MICH.

MONTHS	INSTANCES	CASES RECOVERING	DEATHS	RATIO of INSTANCES to DEATHS.	CASES REPORTED	DEATHS REPORTED as CASES.
1914 JULY.	69	58	11	6.25	59	6
AUG.	99	90	9	11.00	83	2
SEPT.	103	93	10	10.30	98	5
OCT	73	69	4	18.30	56	1
NOV	53	46	7	7.50	39	4
DEC	30	22	8	3.70	25	5
TOTAL for 6 months	427	378	49	8.72	360	23
JAN	19	18	1	19.00	17	1
FEB	18	14	4	4.50	16	3
MAR	26	22	4	6.50	22	2
APR	15	12	3	5.00	11	2
MAY	23	19	4	5.70	19	1
JUNE	20	19	1	20.00	19	1
JULY	47	36	11	4.26	40	5
AUG	73	68	5	14.60	63	1
SEPT.	91	81	10	9.10	86	7
OCT	52	46	6	8.70	51	6
NOV	40	28	12	3.33	34	6
DEC	33	22	11	3.00	30	8
TOTAL for 12 months	457	385	72	6.35	408	43
TOTAL for 18 months	884	763	121	7.30	768	66

FIG. 3.

CHART SHOWING PERCENT OF EACH MONTH'S TOTAL TYPHOID ATTRIBUTED TO SPECIFIC CAUSE. DETROIT BOARD OF HEALTH.

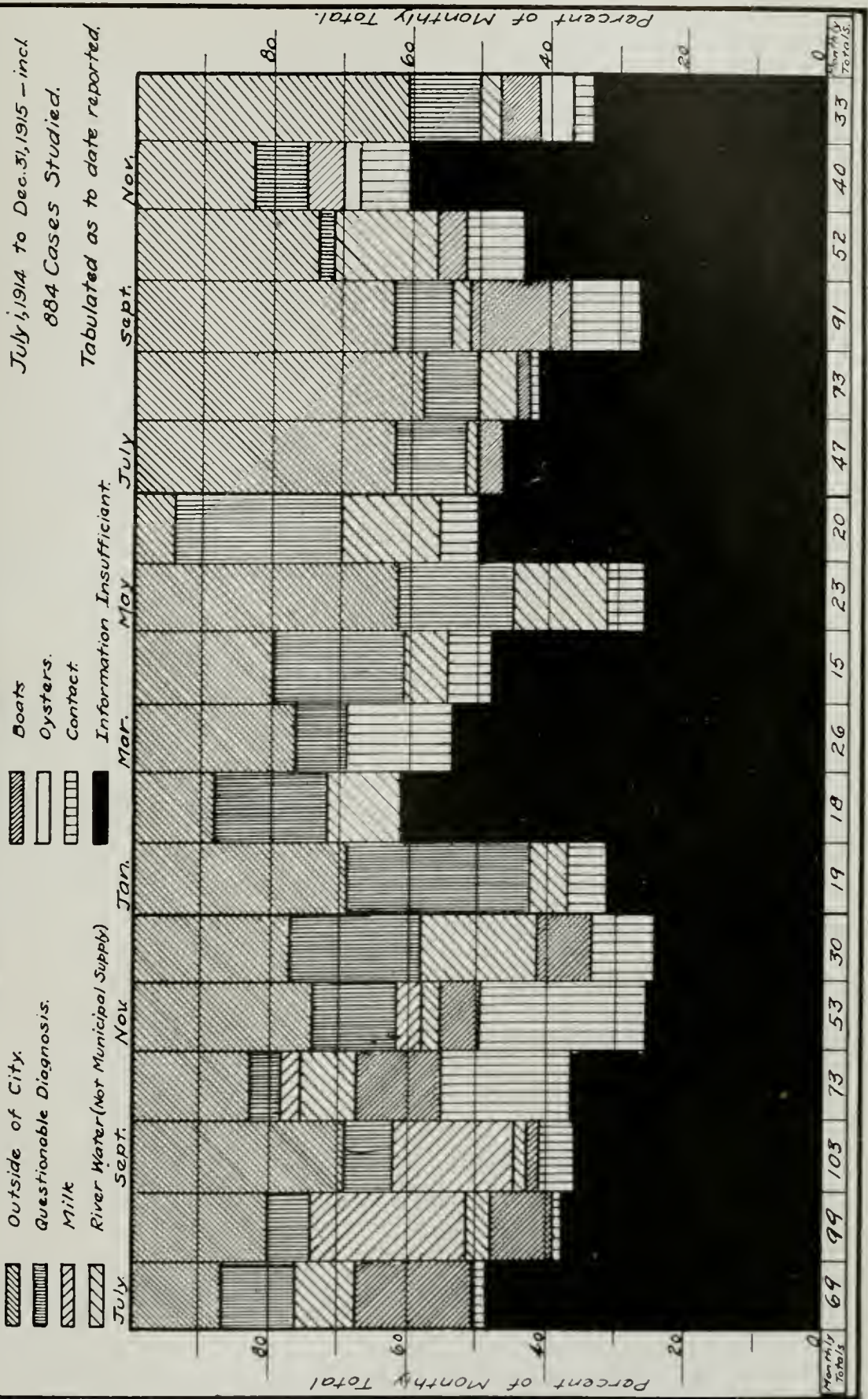


FIG. 4.

It has been our aim to make a detailed analysis of the history of each case and from such a study to draw a conclusion as to the probable source of infection. In 337 cases, or 38 per cent of the instances, we were unable to ascribe any particular source. As in the compilation of mortality statistics it is a difficult problem to select a single cause of death from two or more causes simul-

taneously reported by the physician, it is likewise not a simple matter to assign with certainty a specific source of typhoid fever infection in every instance. Consequently this has been done only in those cases in which the evidence is most conclusive. The accompanying chart (Fig. 4) shows the per cent of each month's total typhoid attributed to each specific source.

Outside.—This refers especially to nonresidents who come to Detroit sick, or who are taken sick at the proper time after their arrival. Lumsden and Anderson report: "As the incubation period of typhoid fever is by no means sharply defined, it is not possible to determine exactly, from a consideration of the element of time alone, on what day or within what period of even ten days the infection has been contracted in a particular case; but, since in the vast majority of cases, certainly, the infection is contracted at some time within a period of thirty days prior to onset of illness and in a considerable majority probably at some time within a period of ten days ending eight days before onset of illness, the place of infection may be determined with a reasonable degree of accuracy in most instances."

This classification would include a case from Royal Oak brought to a Detroit hospital; a person returning sick from a month's outing at some summer resort, and the like. Fig. 4 shows this item to be rather high at those periods when people are returning from their summer homes and their outing trips. The percentage continued rather high throughout the entire eighteen months, due to the number of nonresidents who are brought to Detroit hospitals.

Questionable Diagnosis.—This includes only those cases in which the evidence was most convincing that the patient did not have typhoid fever. It would be an endless task to relate the evidence on which each questionable diagnosis is based since this would require the submitting of numerous letters to physicians, their replies, and the direct evidence of nurses and attendants.

Milk.—Unquestionable evidence of milk infection was found in A's milk during the month of August, 1914, when some 35 cases of typhoid appeared on his route within the city, and many more scattered throughout the villages immediately east of the city line. His milk supply was immediately cut off from the city and the spread of the infection ceased. There was an active case of typhoid on A's farm. Two or three instances of milk infection appeared later on the west side of the city. It is believed that the compulsory pasteurization of all milk, which action took effect in May, 1915, has practically eliminated all possible source of milk infection.

River.—Most of the commercial enterprises situated along the river front have a dual water system, obtaining their supply both from the city mains, this water having undergone the chlorination process of treatment, and directly from the river. Their river intakes are, scarcely without exception, located immediately below some of the large city sewers, consequently the water is highly charged with the colon bacillus, unoxidized organic matter and probably with some specific pathogenic organisms.

The results of a survey made among these factories appear elsewhere in this report. In not a few instances wholesale outbreaks of dysentery among the employees, and many cases of typhoid have been traced to raw river water.

This source of infection was unusually severe during December, 1914, when it was found at one of the manufacturing plants that the employees were provided with river water in all the wash rooms and that the only available supply in the boiler room was from this source.

Boats.—This includes primarily cases from the Marine Hospital, sailors who have been taken sick on boats and removed therefrom at Detroit. There is, of course, some question as to whether this ought not be placed under the heading "Outside" but it has been thought advisable to attempt to subdivide further and charge the boats with their fair portion. Some cases are charged against this class in instances where two or three friends have taken boat trips, afterward developing typhoid, and we have positive knowledge that the boat in question carried a polluted water supply.

During the month of September, 1915, there were reported 14 cases charged against the boats. Twelve of these 14 cases were members of a naval reserve squad which cruised to Erie, Pa., and Buffalo, N. Y. Numerous cases developed in other cities among the members of the crew. The epidemic was investigated by the Public Health Service and the efforts of the Detroit Health Department were confined to the prevention of secondary cases.

Oysters.—During the latter part of 1915 there were reported three cases in which the evidence proved most conclusively that oysters had been the cause of the infection.

Contact.—This item includes unquestionable cases of direct contact such as infection received by brothers and sisters from other members of the immediate family; landladies who have taken care of boarders sick with the disease, etc. It does not include any chance contact by indirect means, as for example, fly contact. Contact appeared but slightly during the summer but found a very prominent place in the autumnal typhoid.

Contact infection is the most common mode in the distribution of typhoid, the medical board of officers in 1898 having placed the percentage at 62.8, while Drigalski, some nine years later, gave for Germany 64.7 per cent. The board further divides contact as follows: direct, 35.01; indirect, 27.79 per cent. It is undoubtedly true that had our information in many instances been more complete, the percentage of contact cases, including indirect, would have been much higher.

Information Insufficient.—This class includes those cases in which we were unable to procure sufficient information to specify any direct source of the disease. However, it is highly possible that had we been able to obtain more history regarding the case, it might have been placed under one of the other seven classifications.

Frequently the wrong address is given when a case is reported, or the "sick" person has moved. Often it was found impossible to converse with the person interviewed or possibly the latter didn't care to understand. In case a death has not previously been reported as a case, it is difficult to obtain sufficient information from the friends or relatives of the deceased.

GEOGRAPHICAL DISTRIBUTION.

Figs. 5 to 11, inclusive, classified as to source of infection, show the distribution of typhoid instances throughout the city. Fig. 5 shows the cases in

which the infection was obtained outside of the city. As would be expected they appear scattered about the more well-to-do sections of the city. A comparative study of the maps showing the distribution of infant births, tuberculosis and

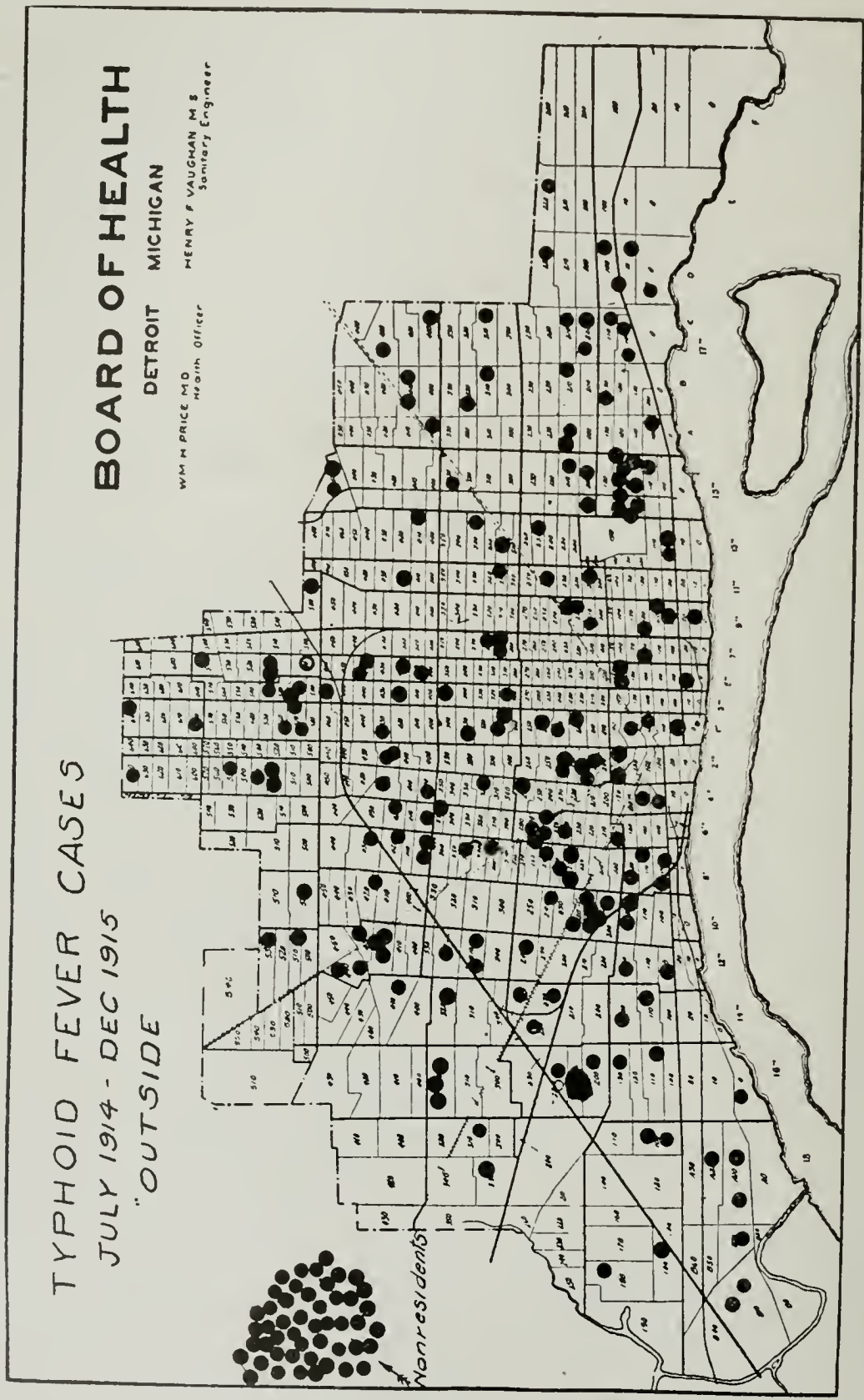


FIG. 5.

pneumonia, shows a decided dissimilarity. Typhoid fever has not been as marked in those districts in which crowded conditions exist—the dirtier districts in which the people are accustomed to remain at home and do comparatively little traveling. These are the districts in which the density of population is the greatest.

Fig. 11 is the one exception to this. During the warm months it appears that a considerable portion of the cases existed in the third, fifth, seventh, ninth and eleventh wards. These were cases which gave no history of having been out-

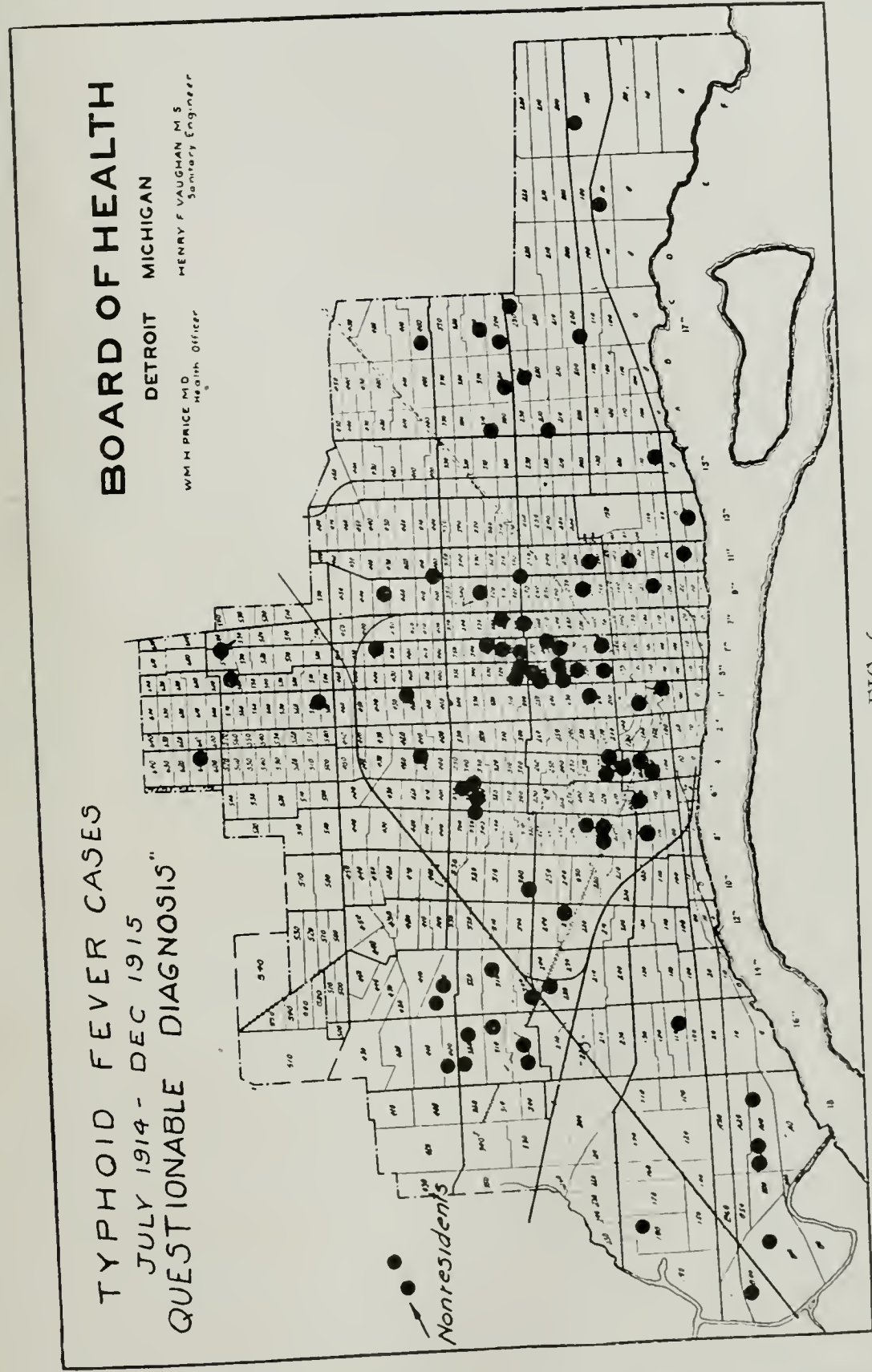


FIG. 6.

side of the city, the information obtained being altogether insufficient to assign any definite source of infection. The prevalence of flies, manure, filthy alleys, uncovered garbage, cock hoppers, and open privies may have played some part in the spread of this infection.

Fig. 7 is of considerable interest in showing the needless havoc that may be wrought by a single milk producer. This shows only a small portion of the cases which resulted from the infection of this milk supply, as many other cases lived in the villages immediately adjoining the city to the east.

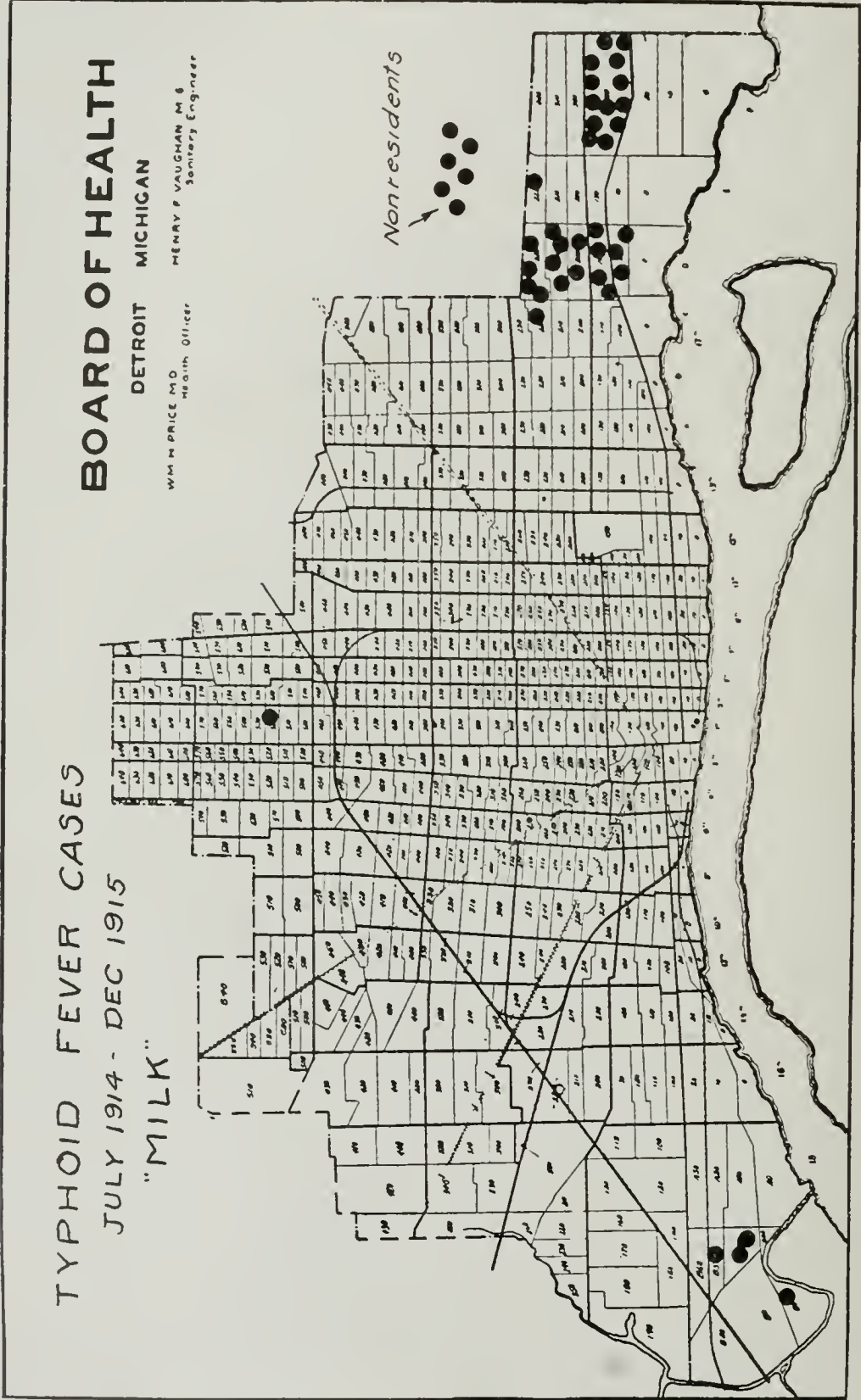


FIG. 7.

SEX.

The accompanying chart (Fig. 12) shows the percentage of cases in males occurring during the first twelve months of the study. As might be expected

the percentage among males is, in general, higher than that among females, probably due to the practice of the former of eating at serve-selves, restaurants or other eating houses away from home. However, during the winter months the

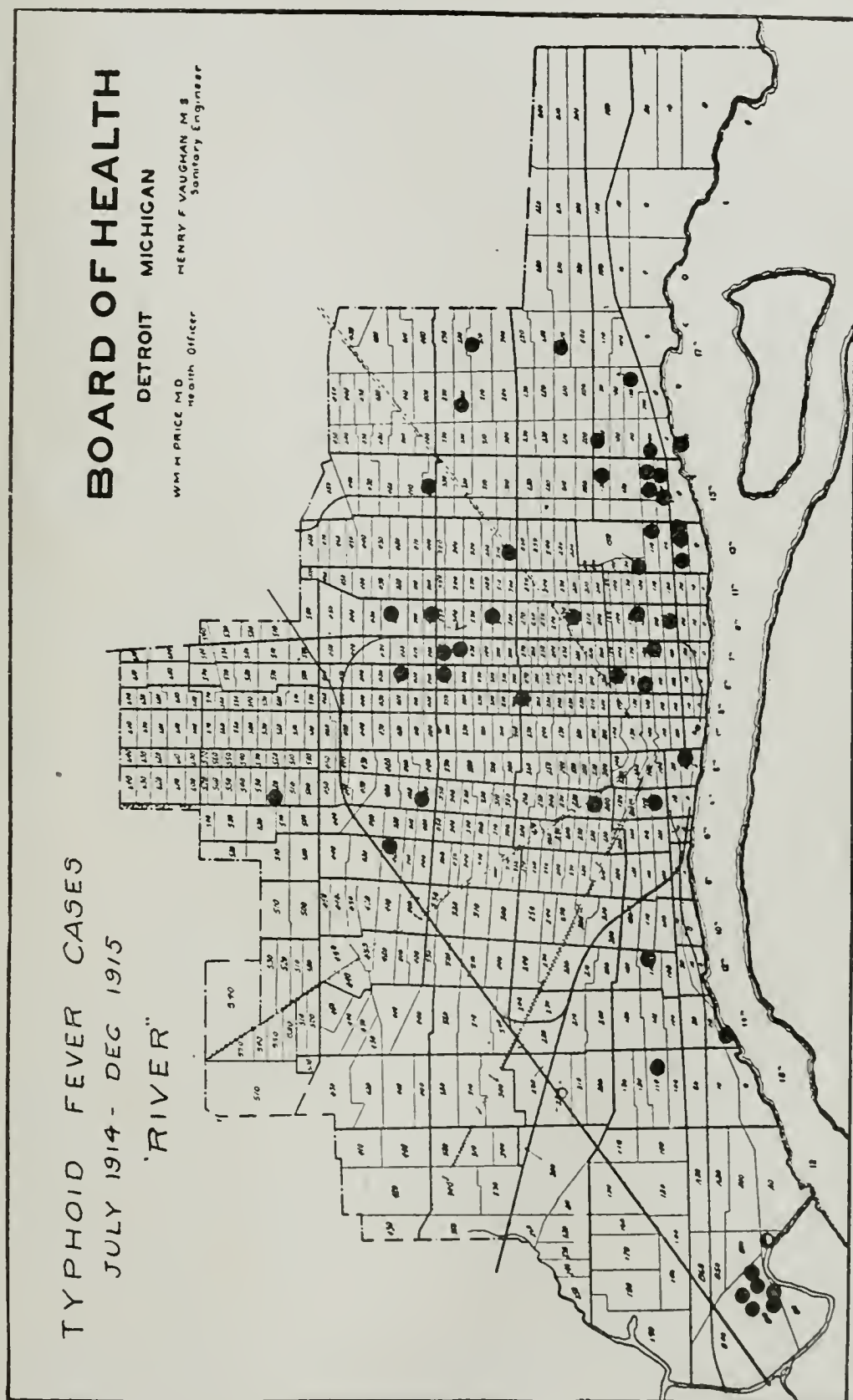


FIG. 8.

number of cases is more evenly divided between the males and females. Possibly some other factor played an important part at this time, some food or drink used pretty generally by both the sexes.

AGE.

Fig. 13 shows the distribution of cases among persons in the different age groups, according to months of onset of illness and sources of infection. Fig.

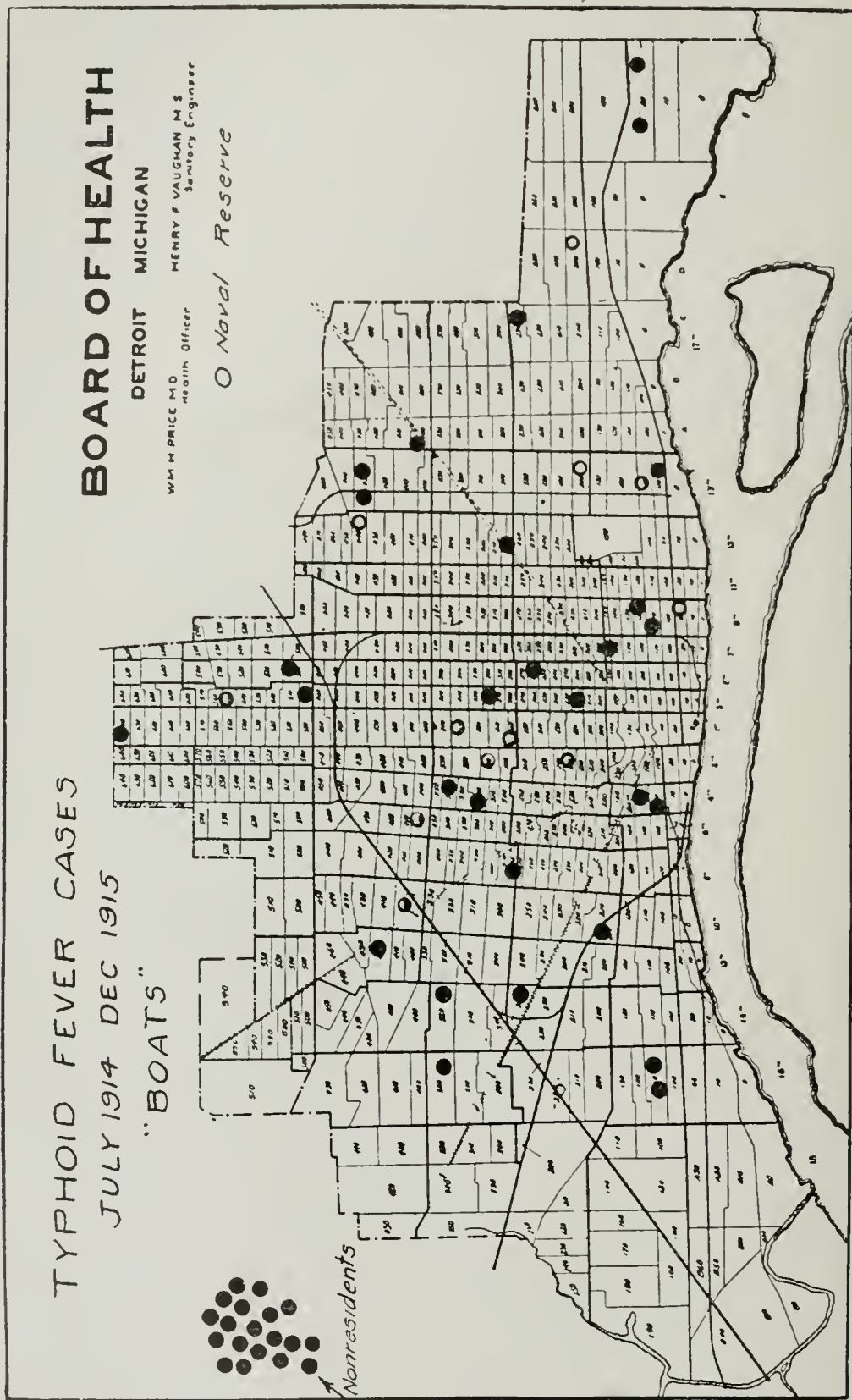


FIG. 9.

14 shows the percentage of cases, by months, furnished by persons in different decades of life.

Of the cases occurring in January, children under ten years of age appear to have furnished 28.5 per cent of the disease. In winter outbreaks caused by

infection conveyed by a public water supply, the percentage of instances among children under ten years of age, who with adults would be equally exposed, has been found to be between 15 and 20. The high percentage would look rather

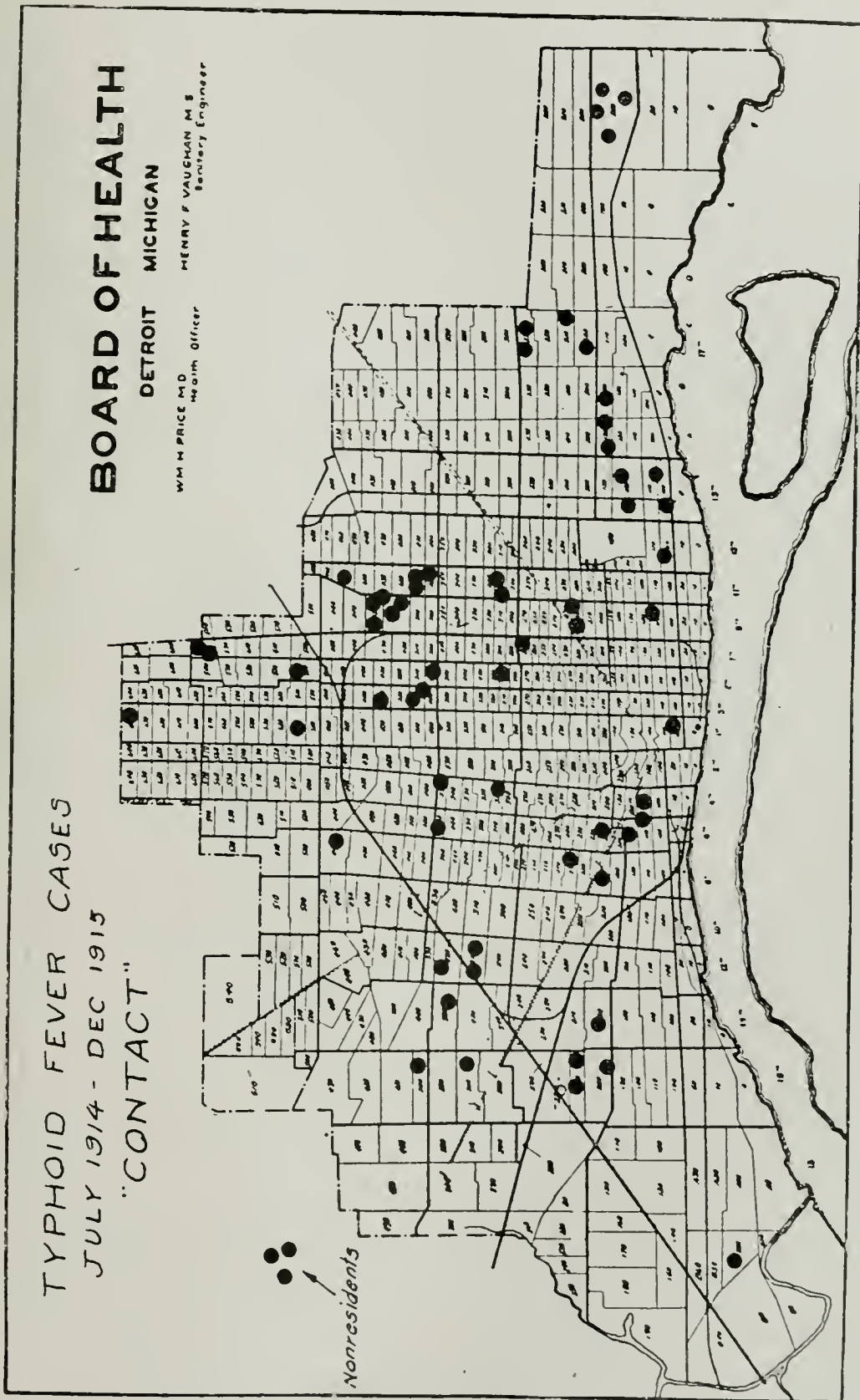


FIG. 10.

suspicious but on further analysis of Fig. 13 we find that of the six cases constituting 28.5 per cent of the January total, 5, or 83 per cent, have been assigned some definite classification. Four of these cases were error in diagnosis which, eliminated, would reduce the percentage at this age group to 9.5 which is far

below the suspicious mark. The percentage of cases among children seems to be rather consistent throughout the year. There is no marked increase during the warm months at a time when they are more subject to general digestive

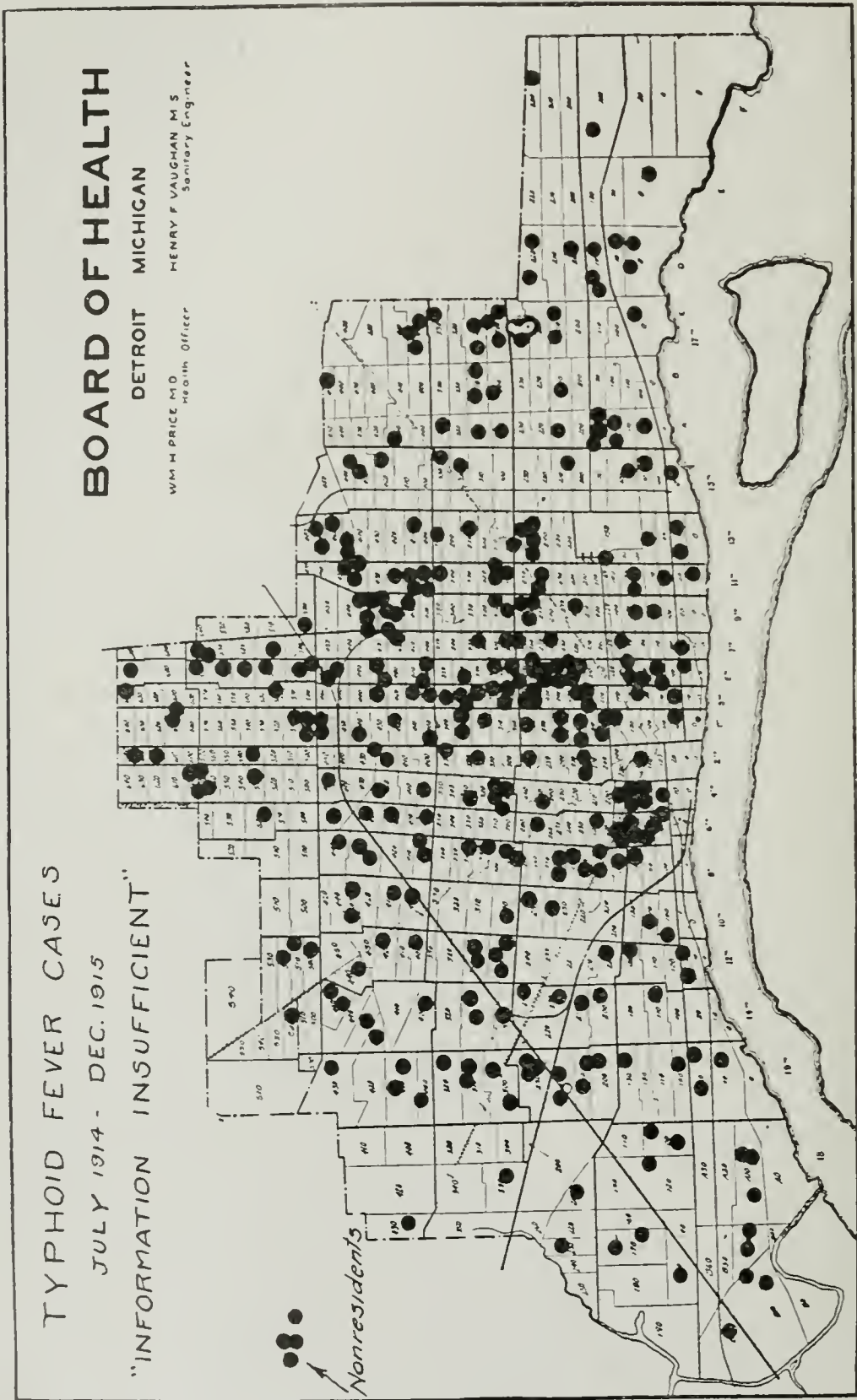


FIG. 11.

disturbances than are adults. Had children been subject to the same infection as adults throughout the year it seems that we might have expected a higher percentage among children during the summer. Evidently there have been some factors especially active during the summer months which have affected

NUMBER OF CASES OF TYPHOID FEVER BY SEX

Month	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Un- Known
Male	15	41	79	46	39	21	14	11	8	8	10	12	4	23
Female	8	27	48	31	24	13	3	10	16	8	5	12	5	7
Total	23	68	127	77	63	34	17	21	24	16	15	24	9	30
% Male	65	60	62	60	62	62	82	52	33	50	67	50	44	77
Month	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Un- Known

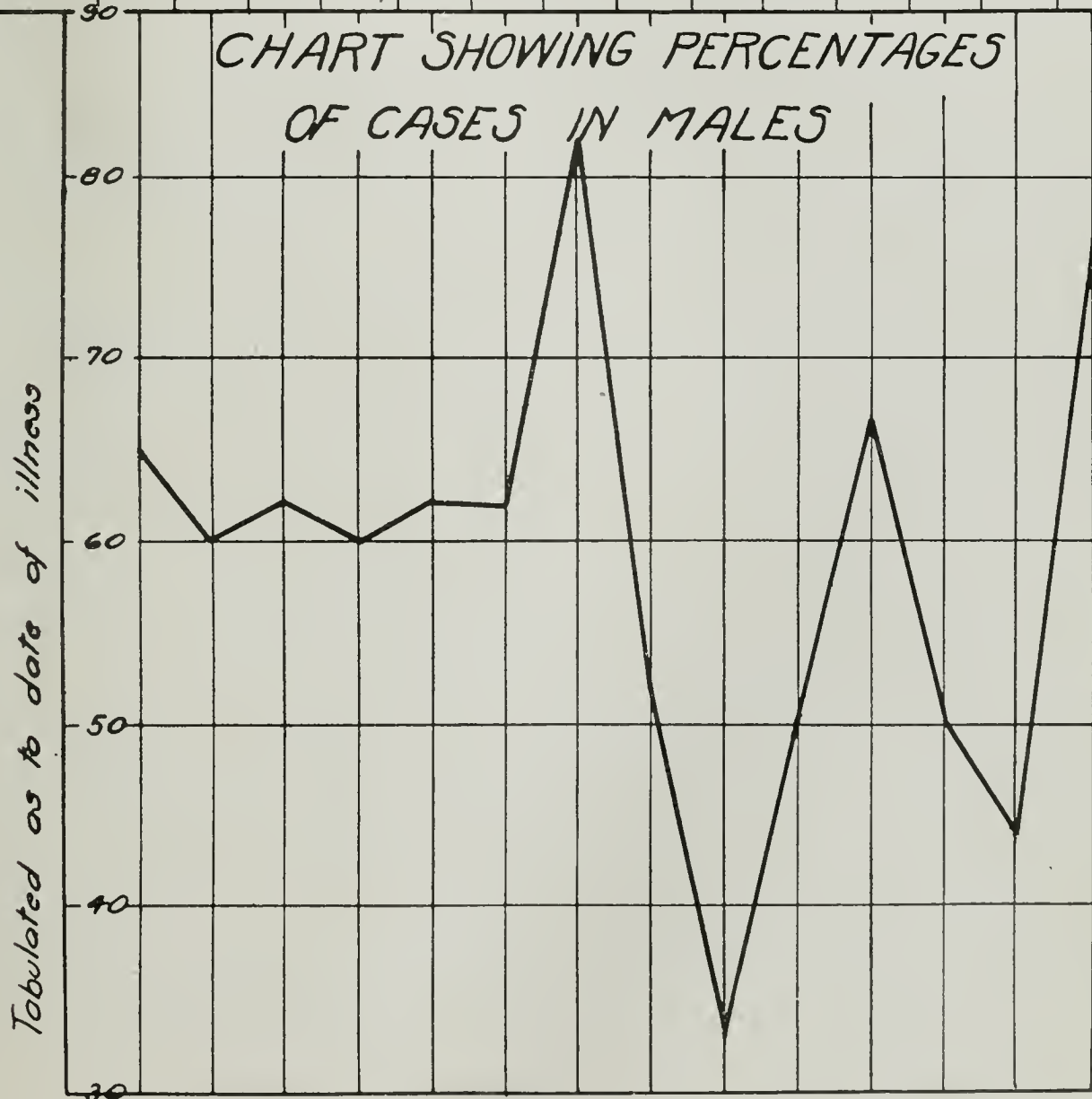


FIG. 12.

only adults. There seem to have been comparatively few people between the ages of 20 and 30 who contracted the disease during March and April. During the remainder of the period this percentage did not change materially.

TYPHOID FEVER STUDIES
BY AGE GROUPS AND SOURCES OF INFECTION.

Key
to
Source.

1. Outside

2. Insufficient Information

3. Other Sources

4. Total

Cases Studied=808

June-1914 thru Dec.-1915.

Age Groups		NUMBER OF CASES TAKEN SICK IN																												Age Groups.	
		June.				July.				Aug.				Sept.				Oct.				Nov.				Dec.					
0-9	-	2	2	4	1	3	2	6	1	7	11	19	2	3	4	9	-	5	3	8	-	-	1	1	1	-	1	2	0-9		
10-19	-	5	-	5	2	9	11	22	10	11	14	35	2	5	3	10	1	6	5	12	3	2	6	11	-	1	-	1	10-19		
20-29	2	2	3	7	6	8	7	21	7	13	13	33	10	10	11	31	7	7	10	24	2	2	6	10	1	4	3	8	20-29		
30-39	1	1	-	2	1	9	2	12	5	6	10	21	4	5	2	11	1	-	6	7	4	-	4	8	1	-	3	4	30-39		
40-49	-	1	-	1	-	1	1	2	3	3	1	7	1	4	3	8	-	3	3	6	-	-	2	2	-	1	-	1	40-49		
50-59	-	-	-	-	-	1	-	1	2	1	1	4	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	50-59		
60-	-	-	-	-	-	2	-	2	1	-	-	1	1	1	-	2	-	-	1	1	-	-	-	-	-	-	1	1	60-		
		Jan.				Feb.				Mar.				Apr.				May.				June.				July.					
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
0-9	1	1	4	6	-	1	2	3	-	1	-	1	1	1	1	3	2	1	-	3	-	1	2	3	1	3	2	6	0-9		
10-19	1	-	-	1	-	4	1	5	2	3	1	6	-	2	-	2	1	1	1	3	1	1	1	3	7	10	1	18	10-19		
20-29	2	6	1	9	1	7	1	9	-	1	1	2	1	-	-	1	4	5	3	12	4	4	5	13	4	6	3	13	20-29		
30-39	-	2	2	4	1	1	1	3	-	1	1	2	1	2	-	3	-	1	1	2	4	3	1	8	2	4	1	7	30-39		
40-49	1	-	-	1	-	2	-	2	1	-	2	3	-	1	2	3	-	2	-	2	2	2	1	5	-	2	1	3	40-49		
50-59	-	-	-	-	-	2	-	2	1	-	1	2	-	1	1	2	-	-	1	1	-	1	-	1	-	1	-	1	50-59		
60-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	1	-	-	-	-	-	-	-	-	60-		
		Aug.				Sept.				Oct.				Nov.				Dec.				Totals.									
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
0-9	4	2	1	7	3	3	3	9	3	-	2	5	3	1	-	4	1	1	1	3	24	36	42	102					0-9		
10-19	9	6	4	19	6	2	6	14	3	4	2	9	-	3	1	4	-	2	1	3	48	77	58	183					10-19		
20-29	10	10	12	32	8	6	5	19	5	10	5	20	3	7	4	14	4	2	2	8	81	110	95	286					20-29		
30-39	3	2	1	6	2	5	1	8	4	3	2	9	-	3	1	4	1	2	1	4	35	40	50	125					30-39		
40-49	5	2	-	7	2	3	3	8	3	-	4	7	1	2	1	4	1	2	-	3	20	31	24	75					40-49		
50-59	-	1	-	1	1	-	-	1	-	3	-	3	1	1	1	3	-	-	-	-	5	12	6	23					50-59		
60-	-	3	1	4	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	2	7	5	14					60-		
																					215	313	280	808							

FIG. 13.

PREVALENCE.

Fig. 15 illustrates the prevalence of typhoid fever divided into the seven classifications which have already been described. The cases are tabulated as

to date of illness, that is, the date on which the patient went to bed, which date can usually be recalled. The time of infection, in most cases, will precede the date of illness from about ten to twenty days.

CHART SHOWING PERCENT OF EACH MONTH'S TYPHOID IN VARIOUS AGE GROUPS

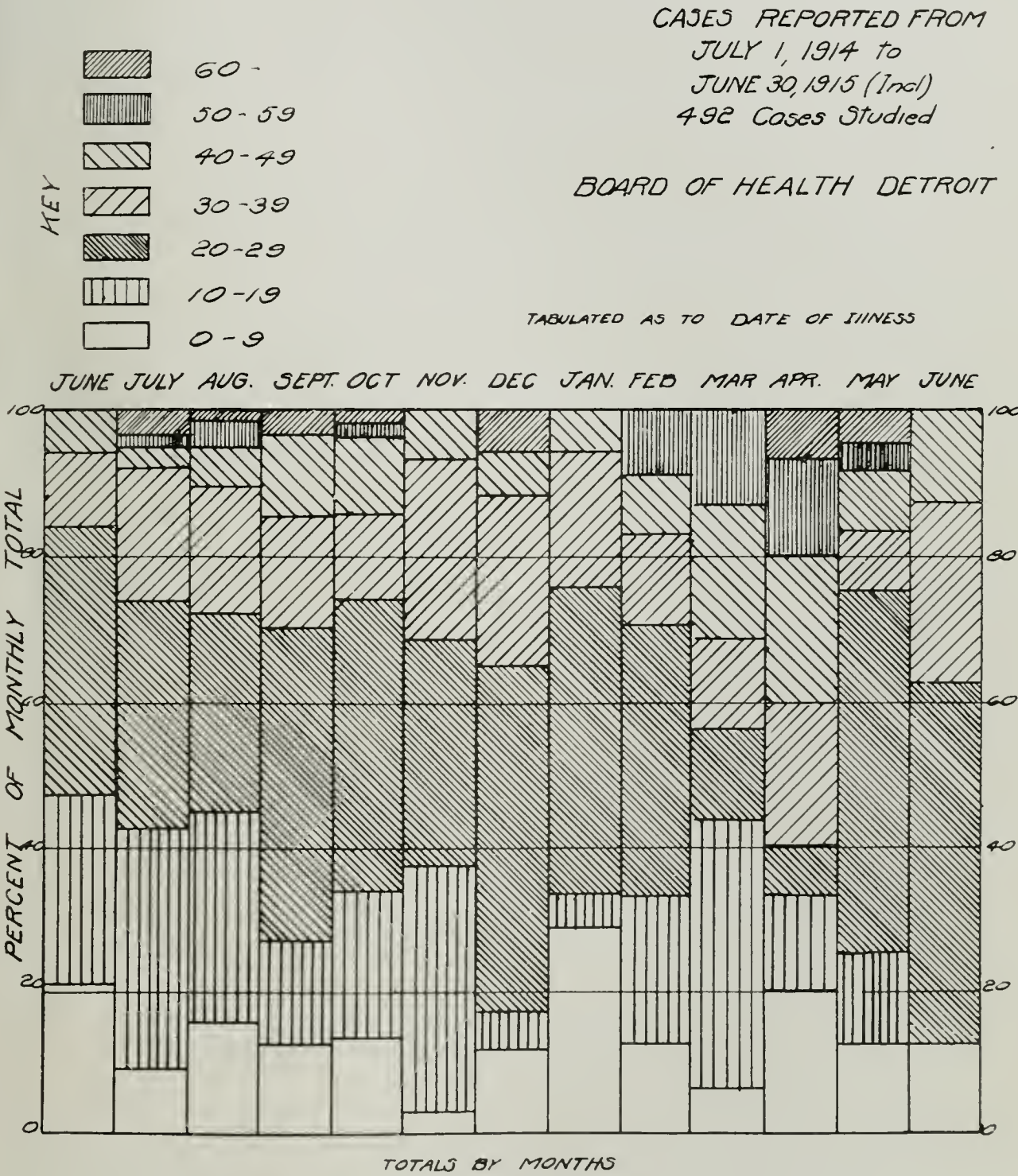


FIG. 14.

Tabulation has been made of typhoid cases by race, occupation, sanitation of homes, etc. Because of the general method which we have adopted in classifying the sources of infection, it seems superfluous to submit these tables at this

PREVALENCE OF TYPHOID FEVER

Key
to Source
1 Outside
2 Questionable Diagnosis
3 Milk
4 River
5 Boats
6 Contact
7 Information Insufficient
1914-1915

Month	Source	Day of Month															Date of Illness															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1914	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
June	4	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	7	-	1	1	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	1	2	-	1	-	-	1	1	1	-	-	
July	1	1	-	1	-	-	-	-	-	1	-	-	-	-	1	3	-	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	1	-	1	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	
	4	1	1	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	5	1	-	-	-	2	1	1	-	-	-	1	-	1	-	2	-	1	1	-	-	1	-	-	-	-	-	-	-	1	-	
	6	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	7	2	3	1	2	-	-	-	1	-	1	1	1	2	1	-	-	-	1	-	1	1	1	1	-	1	3	-	2	-	-	1
Aug	1	2	-	1	2	3	-	3	1	1	-	-	-	3	1	2	-	2	-	3	-	1	1	1	-	-	1	1	1	2	1	1
	2	-	-	-	-	-	-	-	-	1	-	1	-	1	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	3	3	-	-	-	1	-	-	-	-	-	2	-	1	2	3	1	2	2	1	1	2	5	3	1	1	-	1	1	1	-	
	4	1	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	5	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	-	-	-	-	-	1	
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	
	7	1	-	1	-	4	2	4	-	2	-	-	2	1	3	1	1	1	3	-	2	1	3	-	1	1	-	2	1	3	1	-
Sept	1	2	-	-	-	1	2	1	-	-	2	2	-	3	1	-	1	1	-	-	1	1	-	-	-	2	2	-	-	-	-	-
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Oct	1	1	-	-	2	-	-	-	-	-	3	1	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1	
	2	1	2	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	1	
	4	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	
	5	-	1	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	6	1	-	2	1	-	-	1	-	-	1	-	1	-	-	-	-	-	-	1	-	-	-	-	-	2	1	-	-	-	1	2
	7	3	2	1	1	-	-	2	-	-	1	1	-	-	2	-	-	-	-	-	1	-	-	-	-	1	4	-	1	-	-	1

FIG. 15 A.

time. Moreover, very little is known regarding the actual distribution of population by race and occupation as Detroit's growth since the last enumeration by the Census Bureau has been phenomenal. The location of the industry, rather than the nature of the industry, has entered into the problem.

WATER SUPPLY.

Detroit's water supply has at all times been drawn from the Detroit River, not far from the American shore, the first pumping station being located at the

foot of Orleans Street and drawing water 150 feet from shore. This was used until 1877, when the present plant was put into operation, which drew water through three pipes, two of these were 1,500 feet long and 6 feet in diameter,

PREVALENCE OF TYPHOID FEVER

Sheet 2

Month	Source	Day of Month												Date of Illness																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Nov. 1914	1	1	1	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	1	1	1	-	-	-	-	-	1	-	2	-	-
	2	1	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-
	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	1	-	-	1	1	2	-	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	7	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Dec.	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	2	-	-	-	1	-	1	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-
1915 Jan.	1	1	1	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	2	-	1	-	-	-	1	-	-	-	-	1	1	-	-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	2	2	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-	-	1	1	-	-	-	-	-	-	-
Feb.	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	7	5	-	-	1	-	-	1	-	-	-	-	1	1	1	-	-	-	-	-	-	-	2	1	-	1	2	1	-	-	-
Mar.	1	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	4	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	7	1	-	-	-	-	-	-	-	2	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Apr.	1	-	-	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	1	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	1	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	1	1	1	-	1	-	-	-	-	-	-	-	-
May	1	-	-	2	-	2	-	-	1	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	4	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	2	1	-	-	-	1	-	-	-	-	-	1	1	-	1	-	1	-	-	1	-	1	-	1	-	-	-	1	-	-
June	1	1	-	1	-	1	-	-	-	-	-	-	-	1	1	-	2	-	1	-	2	1	-	-	-	-	-	-	-	-	-
	2	-	-	-	1	-	-	-	1	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-
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7	-	-	-	-	-	-	1	-	1	-	1	1	-	2	1	-	-	1	-	-	-	-	-	1	1	-	1	1	-	-	

FIG. 15 B.

the other, 1,000 feet long and 5 feet in diameter. These pipes discharged into a settling basin of 30,000,000 gallons capacity, from which the pumps drew their supply.

The question of location of the intake, in order to eliminate the dangers of immediate shore pollution, has always been given serious consideration. The present intake was located near the northwestern extremity of Belle Isle, after a careful and extensive study of the currents in the lower end of Lake St.

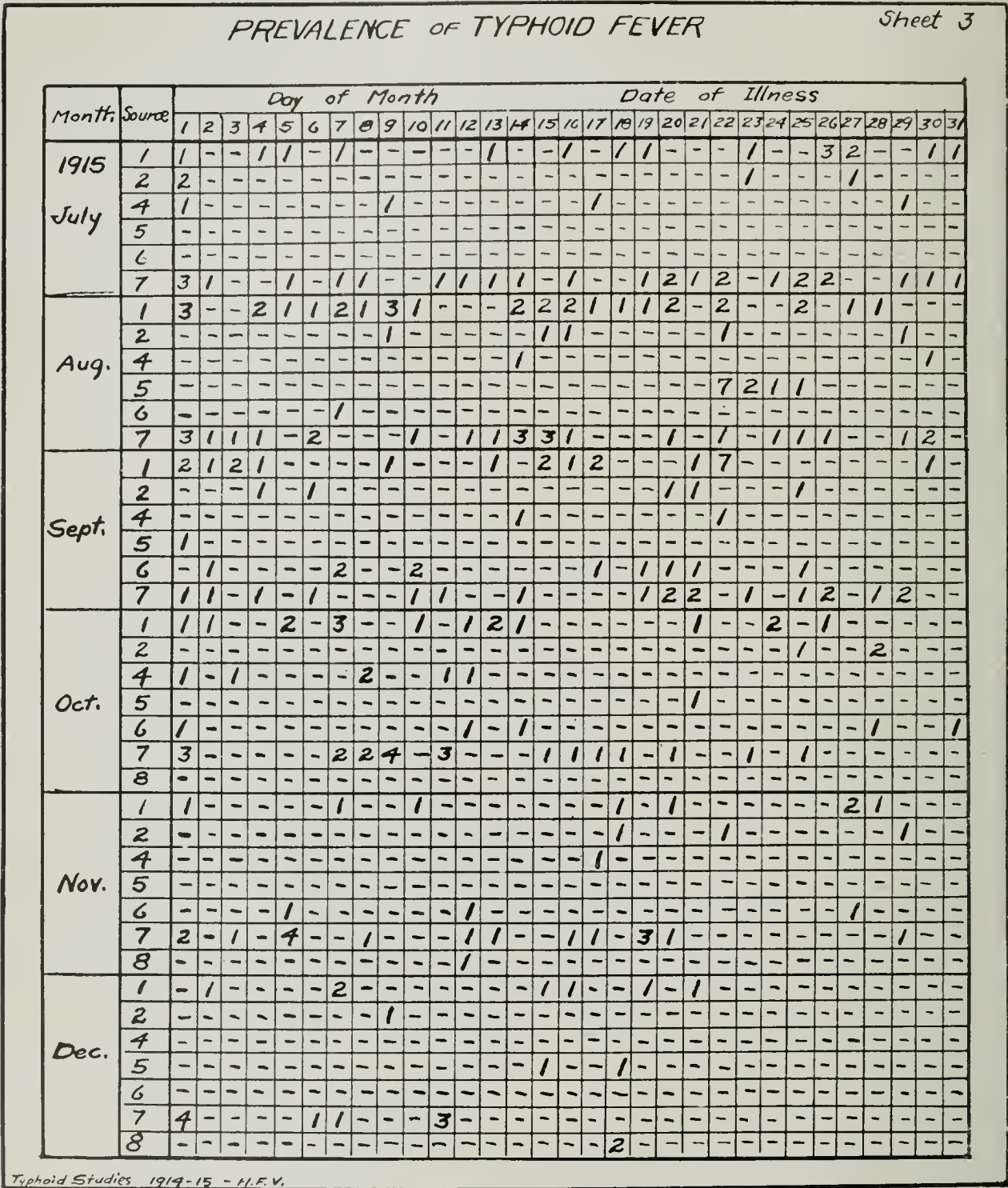


FIG. 15 C.

Clair by Prof. Gardner S. Williams and Mr. C. W. Hubbell in 1897. A ten foot tunnel, 3,000 feet long, connects the crib with the settling basin. This intake was put in operation in December, 1905, and has been in use practically continuously, the old intake being used only in case of extreme necessity.

SOURCE OF POLLUTION OF DETROIT'S WATER SUPPLY.

The water-shed draining into the St. Clair River, Lake St. Clair, and the Detroit River, above the intake, has an area of 5,800 square miles, all of which is very thickly populated. The runoff, owing to the nonporous character of the soil, finds its way quickly to the main water channels, carrying with it its load of surface pollution. Prof. Williams estimates that $1\frac{1}{2}$ inches of rainfall furnishes run-off equal to 20 hours' normal flow of the river past Detroit. The entire sewage of Port Huron and other bordering cities is discharged, untreated, into this drainage system.

The danger from the distant sources is minimized to a large extent by two natural agencies—dilution and sedimentation in Lake St. Clair. The amount of water available for dilution is enormous, and the oxidation resulting must be very extensive. Sedimentation also must play a very important role as is evidenced by the formation at the mouth of the St. Clair River. The two most imminent dangers, no doubt, are the pollution furnished by Connor's and Fox Creeks, and that furnished by Lake Commerce. The danger from the former sources is greatest in times of freshet in the creeks. Increased building operations in this direction, more especially dredging operations, are a potent menace to the water supply.

The U. S. Weather Bureau Observer reports two instances in the last 30 years, in which the velocity of the Detroit River has been as great as two miles per hour in a direction opposite to the normal flow. The effect of such a phenomena will be obvious.

In the year 1913, 37,473 vessels used the Detroit River; not all of these, however, contributed to the pollution in Lake St. Clair. Figures based upon the relative amounts of freight passing Detroit and the St. Clair Flats Canal, show that probably about one-fifteenth of the vessels stop at Detroit, and that about 35,000 use the St. Clair Canal, and hence contribute additional pollution.

The pollution from these two sources is not subject to the natural purification agencies to the same extent as that of the cities, towns, and more distant streams. It is therefore to be considered as a menace to our water supply. A study of the data available may disclose to what extent it is an actual danger.

QUALITY OF DETROIT'S WATER SUPPLY.

Early records of the quality of Detroit's water supply are given under the Report of the General Superintendent of the Water Board for the year 1897. The report is a quotation from the Board of Health analyst as follows:

The importance of a pure water supply to the public health of a community cannot be overestimated. There have been reported from various localities innumerable instances of epidemics traced to the use of a dangerous drinking water. In fact, so numerous are such cases, and so conclusive the evidence pointing to the source of the trouble, that the health authorities of many large cities, appreciating the value of a watchful and preventative policy, have provided for frequent analysis of the public water.

During the past year I have devoted as much attention as possible to our supply, and while the time was not available for as many examinations as seemed desirable, yet several have been made, the results of which, with a brief explanation are here given:

BACTERIOLOGICAL, AND MICROSCOPICAL, ANALYSIS.

April,	1896.	Bacillus typhi abdominalis (typhoid fever bacillus).....	absent
"		Bacillus coli communis	absent
"		Bacteria per cubic centimeter.....	140
		Microscopical—Fine sand, diatomes and algæ.....	...
July	9, 1896.	Bacteria per cubic centimeter.....	95
"	15, "	" " " "	110
"	18, "	" " " "	80
Sept.	22, "	" " " "	200
"	23, "	" " " "	130
"	28, "	" " " "	80
Oct.	27, "	" " " "	40
"	28, "	" " " "	108
"	30, "	" " " "	48
"	31, "	" " " "	130
Dec.	15, "	" " " "	120
"	16, "	" " " "	350
"	23, "	" " " "	85
"	24, "	" " " "	125
"	29, "	" " " "	180
"	30, "	" " " "	180
Feb.	15, 1897.	" " " "	80
"	16, "	" " " "	800
"	17, "	" " " "	320
"	18, "	" " " "	220
"	19, "	" " " "	480
"	20, "	" " " "	530
"	22, "	" " " "	210

The samples were taken from the laboratory tap and subjected to the usual examinations and sanitary and chemical analysis, which includes an estimation of such substances as are of value in determining the drinking quality of the water. The estimation of the number of bacteria is self-explanatory. Good drinking water should not contain more than 1,000 bacteria in each cubic centimeter (about 16 drops).

With the placing of the intake in the main current of the river, in 1905, it was thought that the best available supply was being obtained, and it is reasonable to assume that the quality of the water furnished from this source was materially improved. There is no authentic date, however, relative to the quality of the supply, but we must assume that it remained practically satisfactory until the latter part of 1912 and the spring of 1913, when suspicion was directed toward the water supply by a high typhoid death rate. Because of the situation at this time the Water Board installed apparatus for the addition of calcium hypochlorite to the water. This treatment was begun on April 2, 1913, with the addition of five pounds per million gallons, which was later increased to nine pounds per million gallons in June, 1913. Fig. 16 shows the typhoid death rate by months for the years 1913-1915, inclusive. The arrow indicates the point at which hypochlorite treatment was started. Attention is called to the increase in the typhoid death rate even after sterilization was practiced.

Fig. 17 shows the extent of the B. Coli pollution in the raw and tap water for the last eighteen months. The total instances of typhoid, and the percentage for which the information was not sufficient to assign any specific source of infection is shown above for comparison. There seems for this period to be no direct relation between the number of cases assignable to any specific source and the quality of either the raw or treated water.

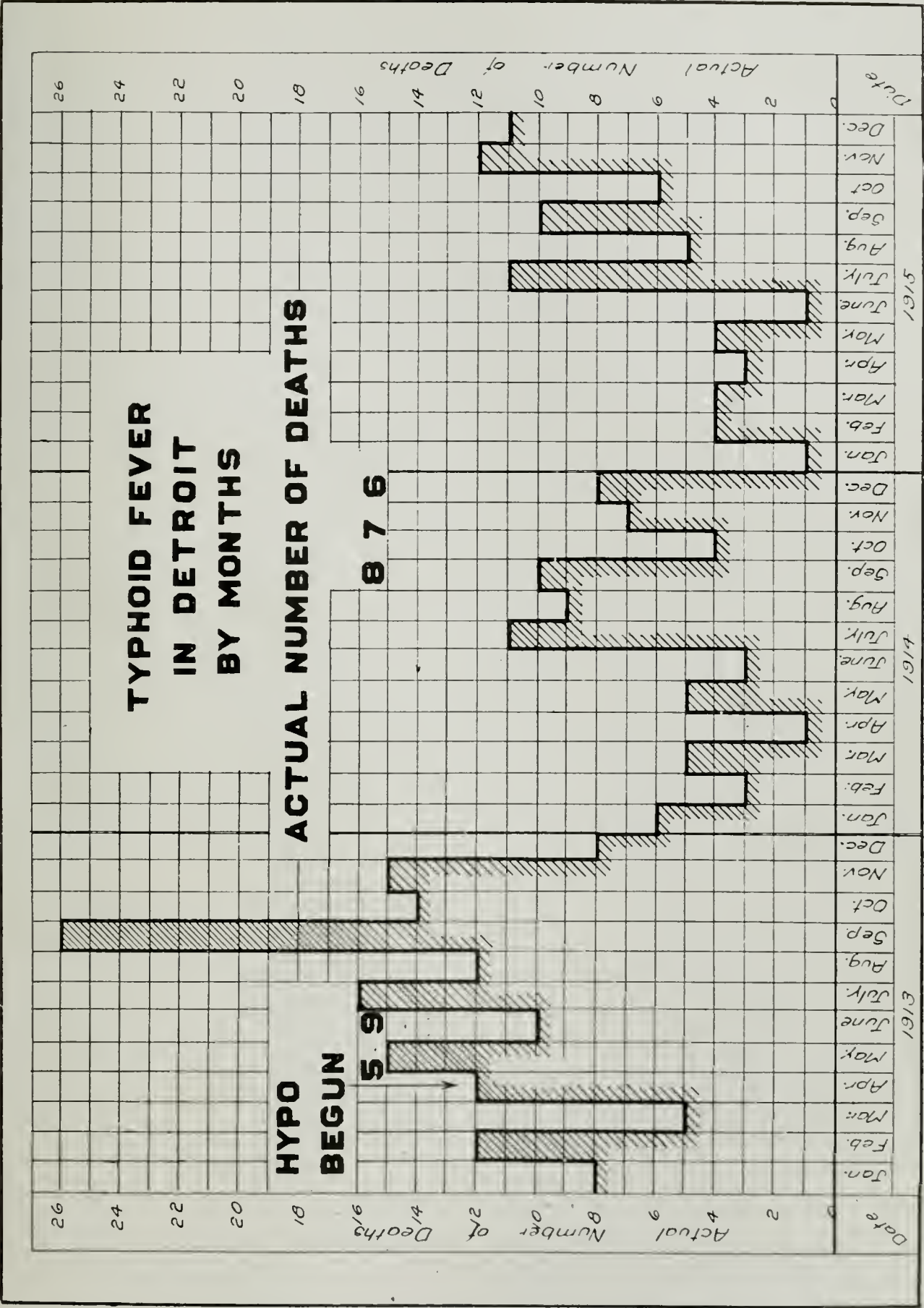


FIG. 16.

Fig. 18 compares the percentage of yearly deaths by months for the last six years with the mean monthly temperatures for the same period. Rather close agreement is shown, in general, between these two curves; two instances may be noticed, viz., April, 1912, and April and May, 1913, when the divergence would lead one to believe that the water supply might possibly be contaminated.

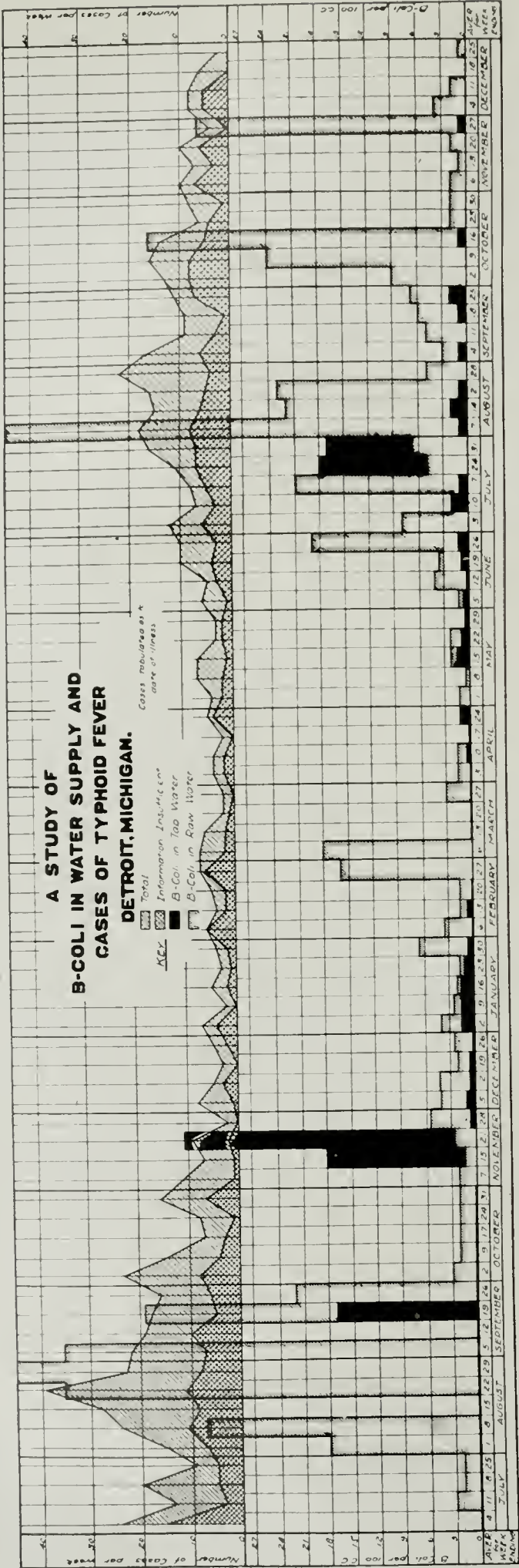
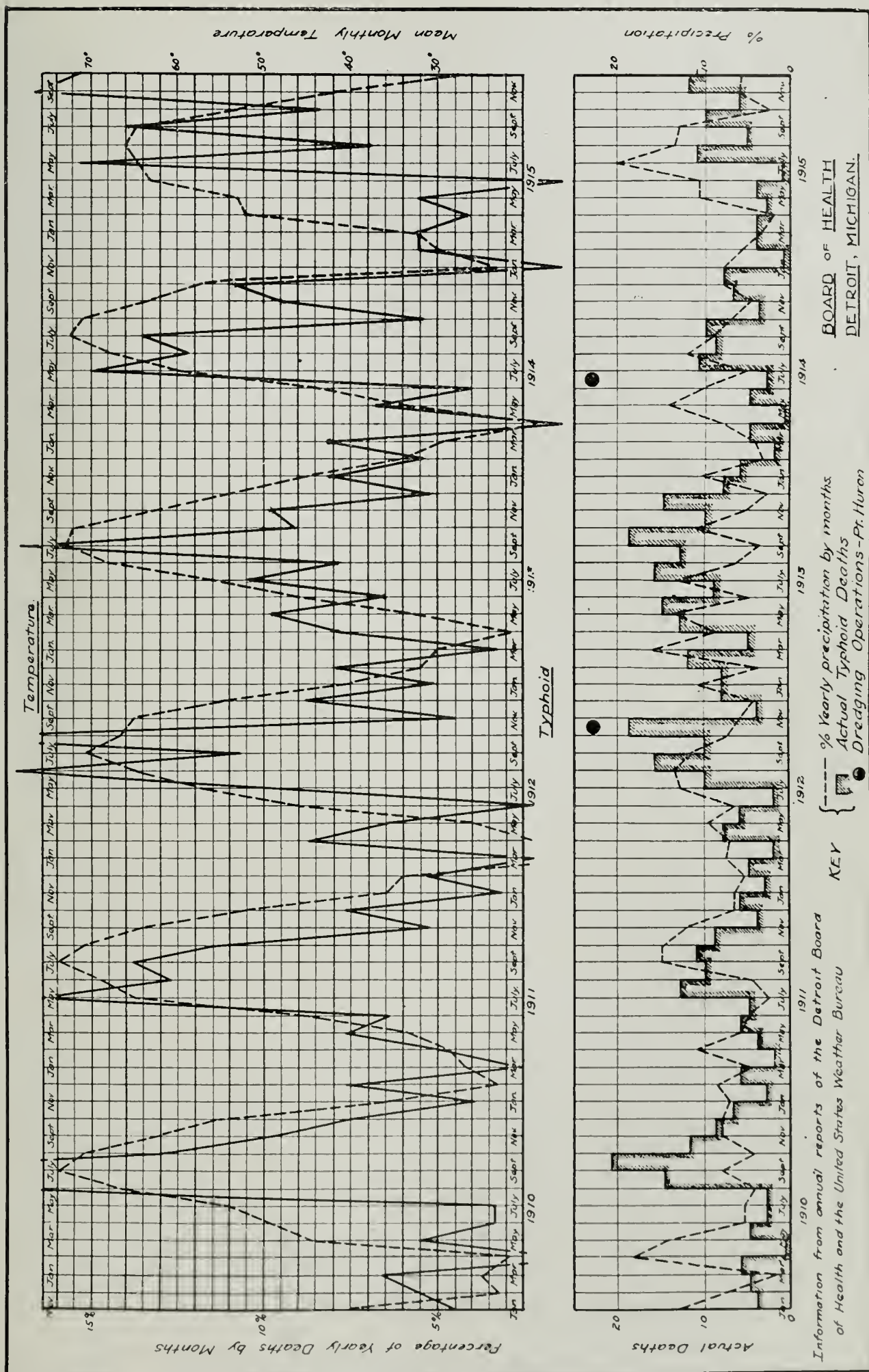


FIG. 17.



On the same chart (Fig. 18) the actual deaths from typhoid are compared with the percentage of precipitation by months.

The use of calcium hypochlorite as a sterilizing agent has been strongly criticised in the city, because of the taste resulting from its use. It is hoped that this objection will be overcome by the use of liquid chlorine, as it is the history of such sterilization that larger amounts of liquid chlorine may be used without imparting an objectionable taste to the water. The change from calcium hypochlorite to liquid chlorine is being gradually made at the present time.

DETROIT SEWERAGE SYSTEM.

Reference to Fig. 1 will show the location of the sewer outfalls of the city. They number about 50 and discharge the sewage into the river without treatment. The sewage from the district above the intake is collected in an intercepting sewer on Jefferson Avenue and discharged into the river below the intake, at the Fairview Sewage Pumping Station. There are, however, instances of sewage being discharged into Connor's Creek. The one most worthy of note is a factory, which is located inside the city limits, and discharges the sewage from 1,000 workmen, together with other factory wastes, into the creek. This is only of recent origin, and steps have been taken to alter the condition.

The entire Connor's Creek Drainage Basin is rather more densely populated than any of the other contributory basins. The portion just to the north and west of the village of Highland Park being practically all platted, there are several instances in which crude sewage is discharged into county ditches which are a part of the Connor's Creek drainage system.

Fig. 2 shows the extent to which the sewage of Detroit affects the general quality of the water in the river below Detroit. The pollution is so great as to render the use of the river, below Detroit, as a source of water supply dangerous, except after very careful and extensive purification. The typhoid death rates of the towns of Wyandotte, Trenton and Monroe, are relatively high, and, no doubt, this gross pollution of the waters adjoining the down river towns and summer resort district is responsible for an appreciable portion of Detroit's deaths from typhoid fever.

MUNICIPAL REFUSE.

The refuse of the city is collected by the Department of Public Works. The garbage and rubbish are collected and disposed of separately. The garbage is required to be kept in proper cans and to be kept free of paper and rubbish. It is collected in all parts of the city at least once in seven days and hauled by one horse wagons to two stations, one at Connor's Creek and Jefferson Avenue East, where it is placed on motor trucks and hauled to the principal station at 24th Street and West Jefferson Avenue, where the garbage is loaded onto cars and taken by rail to French Landing, about 15 miles out of the city, where it is disposed of by a reduction process. The wagons are covered with canvas while being hauled through the streets.

The rubbish is collected in two horse wagons holding about three cubic yards, and is drawn to the various dumps where it is used for filling purposes.

There is always quite a quantity of garbage mixed with the rubbish, which renders the dumps a breeding place for flies. The work of the antifly campaign during the last summer, however, has done much toward improving this condition by bringing about a better separation of garbage and rubbish.

MILK SUPPLY.*

Previous to the year 1910, several outbreaks of typhoid fever occurred in Detroit due to infected raw milk. These outbreaks usually gained considerable headway before being discovered, there being no system employed whereby the cases came to the attention of the Health Department until several deaths from typhoid occurred in one locality.

Up to this time the most notable outbreak of milk-borne typhoid occurred during the year 1908. At this time an east side milk dealer was noticed to have a number of cases appearing among his customers, after several deaths had occurred. A canvass of his milk route was made by inspectors of the Board of Health and over one hundred cases found. This dealer was drawing most of his milk supply from farms adjoining Connor's Creek. Investigation of these farms showed that nine were housing typhoid patients. The milk from these farms and all others directly bordering on this creek was excluded from the city. The milk dealer's premises and all his appliances and utensils were very thoroughly disinfected and sterilized before he was again allowed to distribute milk.

Connor's Creek was found to be very badly polluted with sewage, it being a natural drain for the east end of Wayne County. Practically all farms bordering on this creek used it for watering their live stock.

Other outbreaks occurred from time to time up to the year 1910, but were always checked before they gained any considerable headway.

During the year 1910 the milk inspection division was reorganized and the number of inspectors increased. At this time new regulations were adopted, requiring the milk dealer and milk producer to notify the Health Department of any contagious disease which might exist in his family or in that of his employees, or on any of the farms from which he drew his daily milk supply.

Through the cooperation of the dealers and producers no more explosive outbreaks occurred until the summer of 1914, when a milk dealer, vending milk in the city from a dairy outside, deliberately concealed a case of typhoid fever in his family. The patient was removed to a city hospital, from which place the case was reported. On investigation, the visiting nurse was informed by the patient that she had come in from the country and that her people were not engaged in the milk business. About this time other cases were being reported and charged against this milk dealer, the father of this patient. Further investigation by the milk inspection division revealed the above facts. The dealer was eliminated from Detroit, leaving a trail of eighty cases of typhoid and six deaths.

Shortly after this outbreak all milk sold in Detroit was ordered pasteurized. Since the enforcement of compulsory pasteurization May 1, 1915, no cases of

*We are indebted for the following information to Mr. Krehl, Chief Milk Inspector, Detroit Board of Health.

typhoid or other contagious disease have been traceable to the milk supply. However, milk dealers and producers are still obliged to report contagious diseases as mentioned above.

FACTORY WATER SUPPLIES

In the spring of 1914 the attention of the Health Department was attracted by the large number of typhoid cases reported among workmen at one of the large river front factories. An investigation developed the following points as shown diagrammatically in Fig. 19.

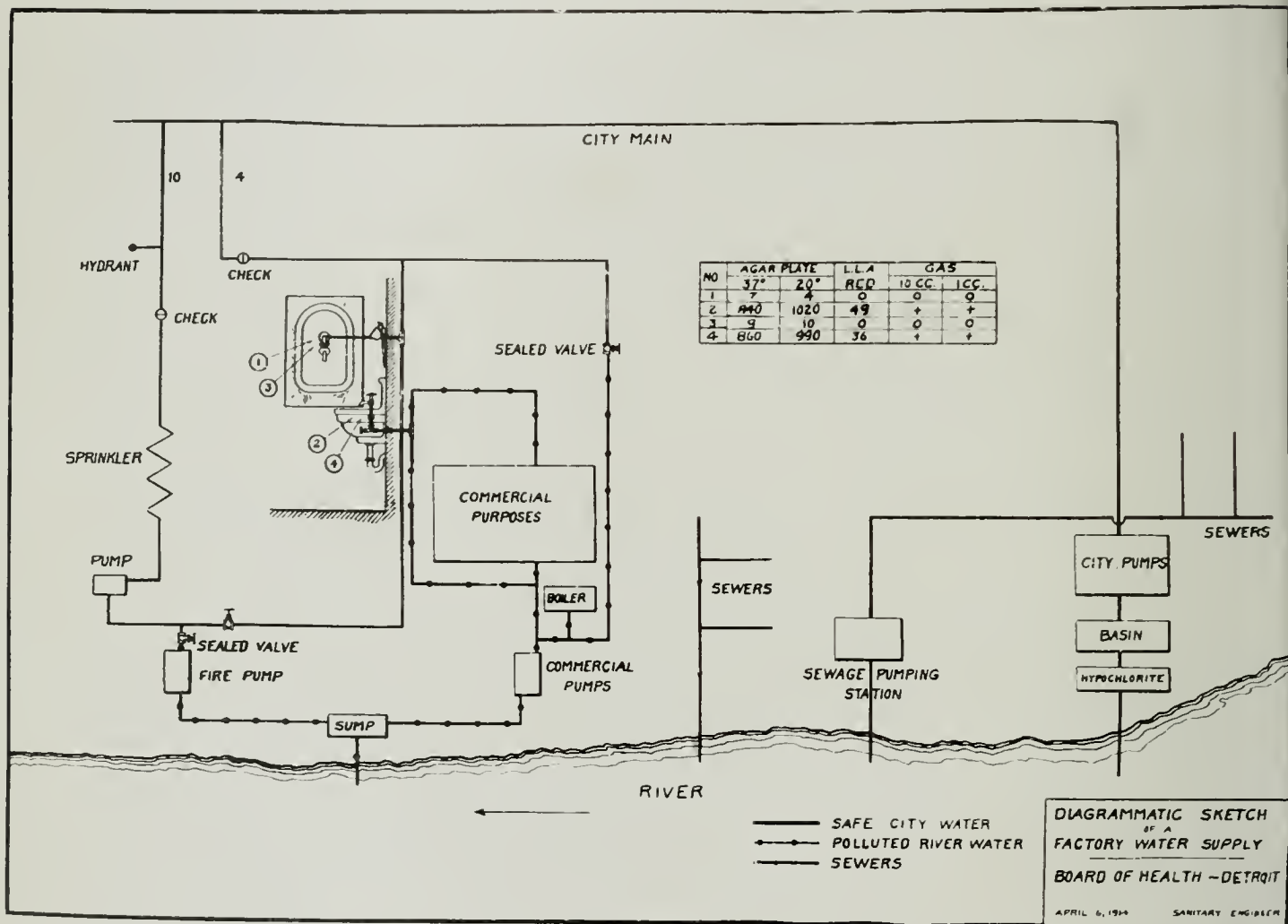


FIG. 19.

The factory in question was using raw river water for boilers, washing rolls, flushing toilets, lavatory, and on the sprinkler system. As is shown in the chart, the relative position of the drinking fountain tap, supplied with city water, and the lavatory, supplied with river water, was such that workmen could easily use the river water for drinking purposes, if for any reason the city supply was not easy of access, there being nothing to indicate that one was suitable and intended to be drunk, and that the other was grossly polluted. Moreover, the very use of the river water in the lavatories constituted a danger and a menace to the health of the employees, as it is only a short time when the typhoid bacilli are on the face and hands until they are in the mouth and into the alimentary canal.

The finding of this condition was the beginning of a survey which included

all the river front factories, which were drawing any part of their water supply from the river, and the subsequent issuing of an order, based upon the results of this survey, to the effect that wherever water was used in such a manner that the workmen must come into intimate personal contact with it, the water must be of a quality equal to that furnished by the city main. The manufacturers have been quick to see the need of such a measure, and compliance is practically unanimous, although some time is needed to complete the alterations in piping made necessary, the cost being estimated in some instances to be three and four thousand dollars.

WATER SUPPLY OF BOATS.

The passenger boats operating out of Detroit carry annually, approximately 10,000,000 passengers; about six million of these are carried on those boats termed "ferries," and operating wholly within the Detroit River, between Detroit, Belle Isle and Windsor. The boats carrying the remaining four million operate in the river, and the lakes and rivers adjoining it on either end.

A survey by the Health Department, during the summer of 1914, proved beyond doubt, that the water supplied to the passengers for drinking purposes, on all the boats operating out of Detroit, was far below that standard of bacteriological purity which indicates a safe drinking water. At the time of the survey, the boats were, without exception, drawing their supply from the lake or river water through which they passed, and were supplying it to their passengers without treatment.

Fig. 20 shows diagrammatically the general arrangements for supplying drinking water as they were found at this time. The fresh-water storage tanks were filled by the general service pumps, when the boat was in the best water attainable. There was always the danger that the pumps had been contaminated by the water drawn in while laying at docks, and that this contamination still remained to further pollute the supply. Then, too, there is the possibility of leaking valves, which might allow water to pass into the freshwater tank when the decks were being washed, or when the trim tanks were being filled; no instance being found in which there were two valves on the same line with a drain between.

It was shown by the Progress Report of the International Joint Commission in reference to the Pollution of Boundary Waters * * * Date of January 16, 1914, that in all instances, the lake and river waters traversed by the vessels, considered here, were of such character as to be rendered suitable for drinking purposes only after extensive purification.

Upon completing this survey, the different navigation companies were notified of the results, and the conditions as they existed on the boats in question were pointed out, it being plainly demonstrated that it was to the ultimate advantage of the navigation company to supply its patrons with a safe water. Assurance was given that full cooperation might be expected from the Health Department.

Because of the peculiar conditions affecting the purification of water on boats, the Health Department did not feel in a position to dictate as to how the purification should be brought about; but simply maintained the policy that water

DIAGRAMATIC SKETCH OF THE
WATER SYSTEM USING LAKE WATER
WITHOUT TREATMENT.

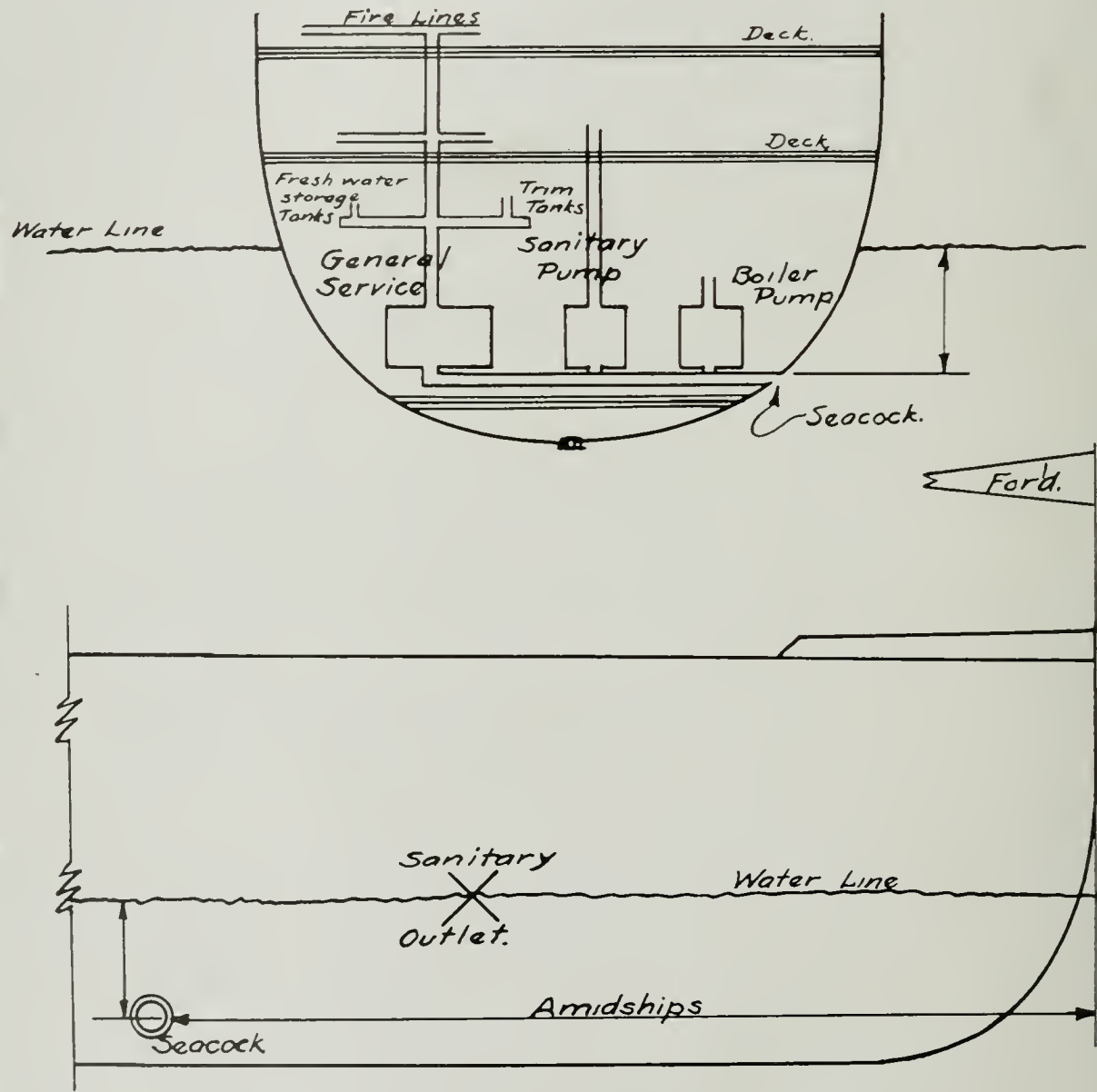


FIG. 20.

was to be considered safe which came within the limit of permissible impurity established by the Treasury Department, that is to say, the water supplied for drinking purposes must not show the presence of more than 100 organisms per

cubic centimeter when plated on standard agar and incubated for 24 hours at 37°, nor may it show the presence of the Colon Bacillus in more than one of five 10 c.c. portions by the fermentation of standard lactose broth within 48 hours at 37°, and the subsequent confirmation of the same on Endo's media and by refermentation. This desired end was sought by four distinct methods, viz.:

1. Taking water from city mains.
2. Taking lake water and filtering by a rapid sand filter.
3. Taking lake water, filtering by a rapid sand filter and treating the effluent by liquid chlorine.
4. Taking lake water and sterilizing it by means of live steam under pressure.

Fig. 21 shows the results of analyses of samples of water by *Method No. 1*, collected during the past season. These boats drew their supply from a 2 inch line on the dock, using a hose connection to fill the tanks located in the hold. The high count in these samples is undoubtedly due to incubation in the tanks, the temperature being usually between 85 and 90° F. Samples of tap water left standing at room temperature for a period of 24 hours showed an increase in the count as follows:

Original count,	13;	final count,	2,400;		
"	"	75;	"	"	innumerable.

This seems to indicate that the high count was simply a multiplying of those forms already in the water. Sterilization of the tanks by a hot solution of calcium hypochlorite was recommended as a preventive measure.

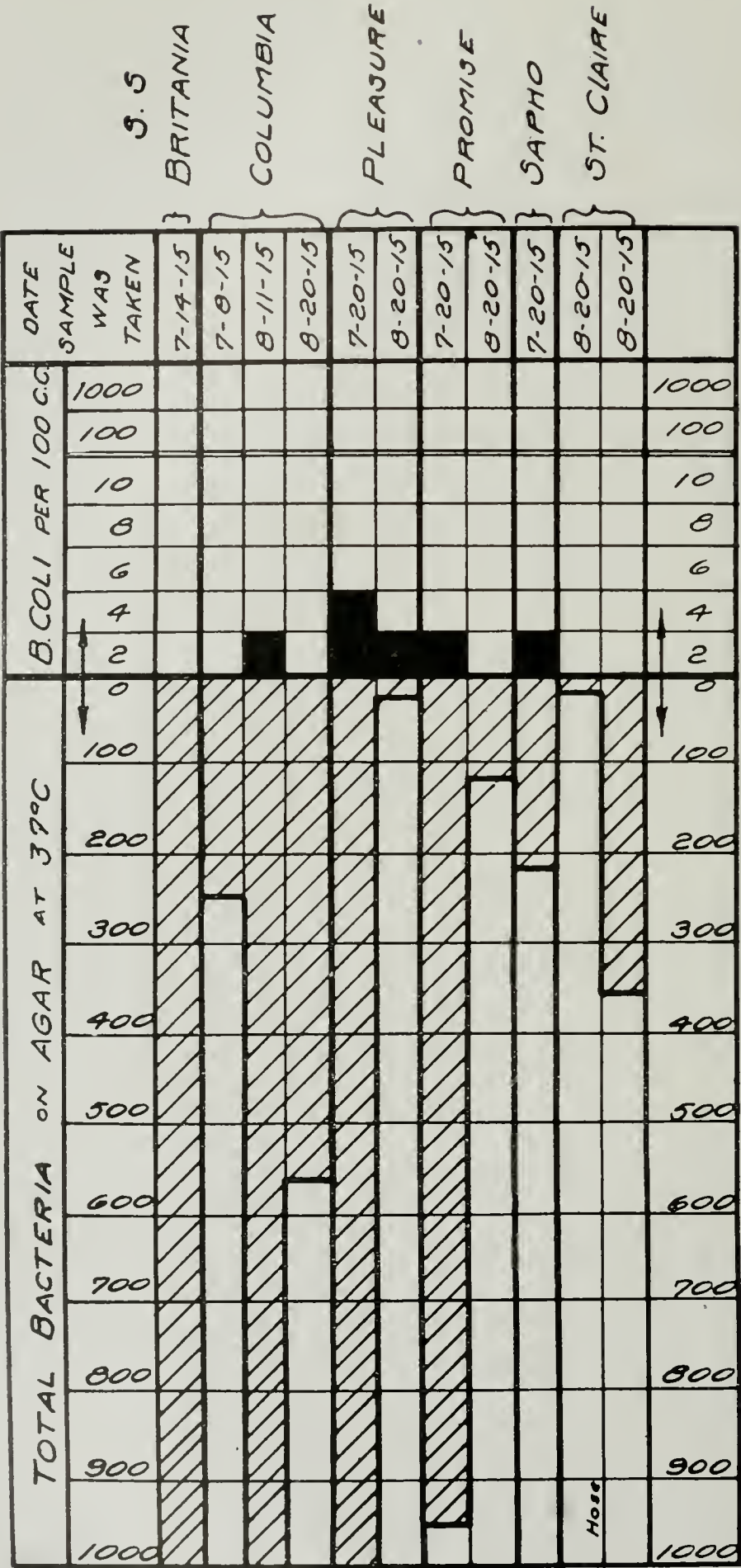
Method No. 2 consists in the application of the pressure type of rapid sand filter. The first difficulty encountered in this method of purification was to find a place in which to put the filter. It was finally located in the engine room near one of the cylinders. The same condition of high temperature also obtains here.

Fig. 22 shows the total bacterial count in all the samples taken since the first of the season. A remarkable decrease toward the last is due largely to the untiring efforts of the engineer in charge, in cleaning and sterilizing both the lines and storage tanks.

Fig. 23 gives the colon content, as well as the count, for the latter part of the season. Due to the change in the method of analysis, the record of the B. Coli for the whole season is not directly comparable. It is evident, however, that even with the exercising of great care in the operation of a rapid sand filter, it cannot be relied upon to furnish a safe water when the character of the source is such as the boat in question traversed during the past season. The failure to supply water of a suitable character is probably due to high temperatures surrounding the filter and inability to wash the filter with filtered water.

Method No. 3 consists in filtering lake water through a rapid sand filter and treating the effluent with liquid chlorine. The same difficulties were encountered in the operation of the filter in this instance as occurred in the one already discussed.

The liquid chlorine is used to make up a solution of chlorine water of known strength, which in turn is fed into the main supply pipe in quantities propor-



D, B I & W FERRY CO:

FIG. 21.

D. F.

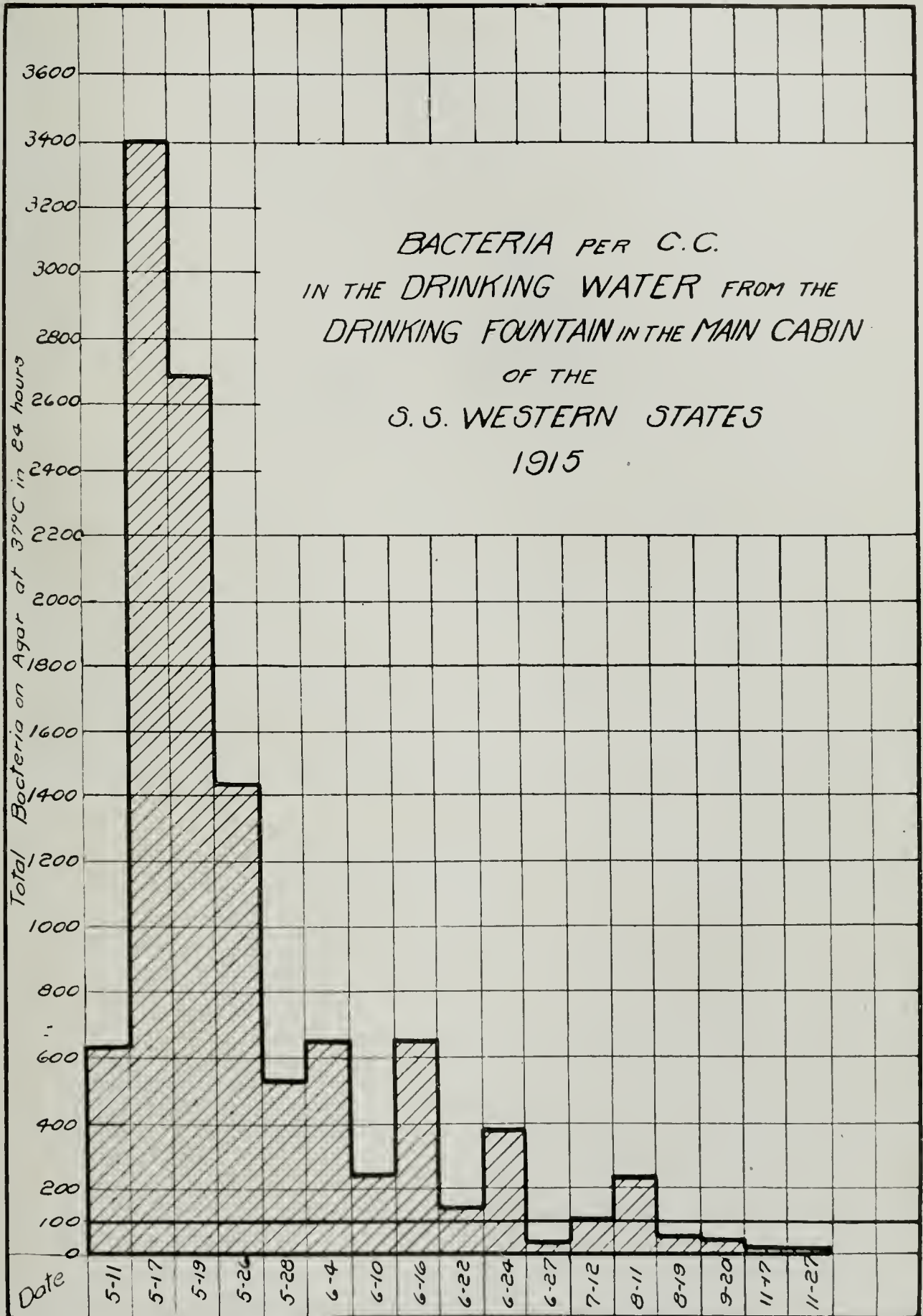


FIG. 22.

tional to the demand, by means of a shunt feed around a constriction; being an application of the Venturi principle, one charge of chlorine being sufficient for about four days' use.

Fig. 24 shows the results obtained by this method. Examination of the results in the first column shows that the general character of the raw water was very low, while in only three instances does the final product fail to meet the requirements of the standard. From the results obtained, it would seem at first glance that this method would be one worthy of consideration, but further investigation discloses that the operation of the chlorinating apparatus is complicated, and so far out of the line of work of the ordinary engineer in charge of a lake steamer that the chances of its being operated satisfactorily on all vessels are very slight. The duties of the engineer are primarily with his engine, and in the time of crisis, the water supply would be given only little attention, and due to the nature of the chlorine gas serious conditions might arise should the apparatus develop a leak. In the vessel in question a situation quite similar to the above did occur, after one of the hard rubber connections between the absorption chamber and the chlorine water storage chamber had broken during a hard storm. The engineer, in charging the apparatus, had drawn the required amount of gas into the measuring chamber and was about to absorb it when the broken connection was noticed by the escape of gas. The engineer had the presence of mind to turn on the water and flood the room, absorbing the gas and preventing any disastrous results. With a more serious break or a larger quantity of gas, the situation would have been dangerous.

Method No. 4, that of sterilization by steam under pressure, was developed by Mr. Winfield Dubois, Chief Engineer of the White Star Line, and consists essentially of heating the water to a temperature above the thermal death point of the pathogenic organisms contained and cooling it again, maintaining a pressure at all times of five to ten pounds. Inasmuch as the water is not exposed to the air during the time it is heated above the boiling point, there is no opportunity for expelling the entrained gases, so that the treated water does not have the flat taste characteristic of a boiled water.

Fig. 25 shows diagrammatically the device as installed on the Steamer Owana:

- A. Injector for drawing water from seacock and forcing it through the system.
- B. A jet for introducing live steam under pressure.
- C. A tank containing 208 feet of $1\frac{1}{2}$ inch pipe for cooling purposes.
- D. Pump for circulating cooling water.
- E. Storage tank.
- F. and G. Control thermometers.
- H. Blow-off valve.

When the device is operated the blow-off valve is opened and the system from the steam jet is blown out with live steam for five minutes. The injector is then primed and allowed to pump to waste through the blow-off until the temperature of the water delivered, as shown by Thermometer *F*, is properly regulated by the steam jet. When the desired temperature has been reached the cooling pump is started and the blow-off closed, so that water is delivered into the

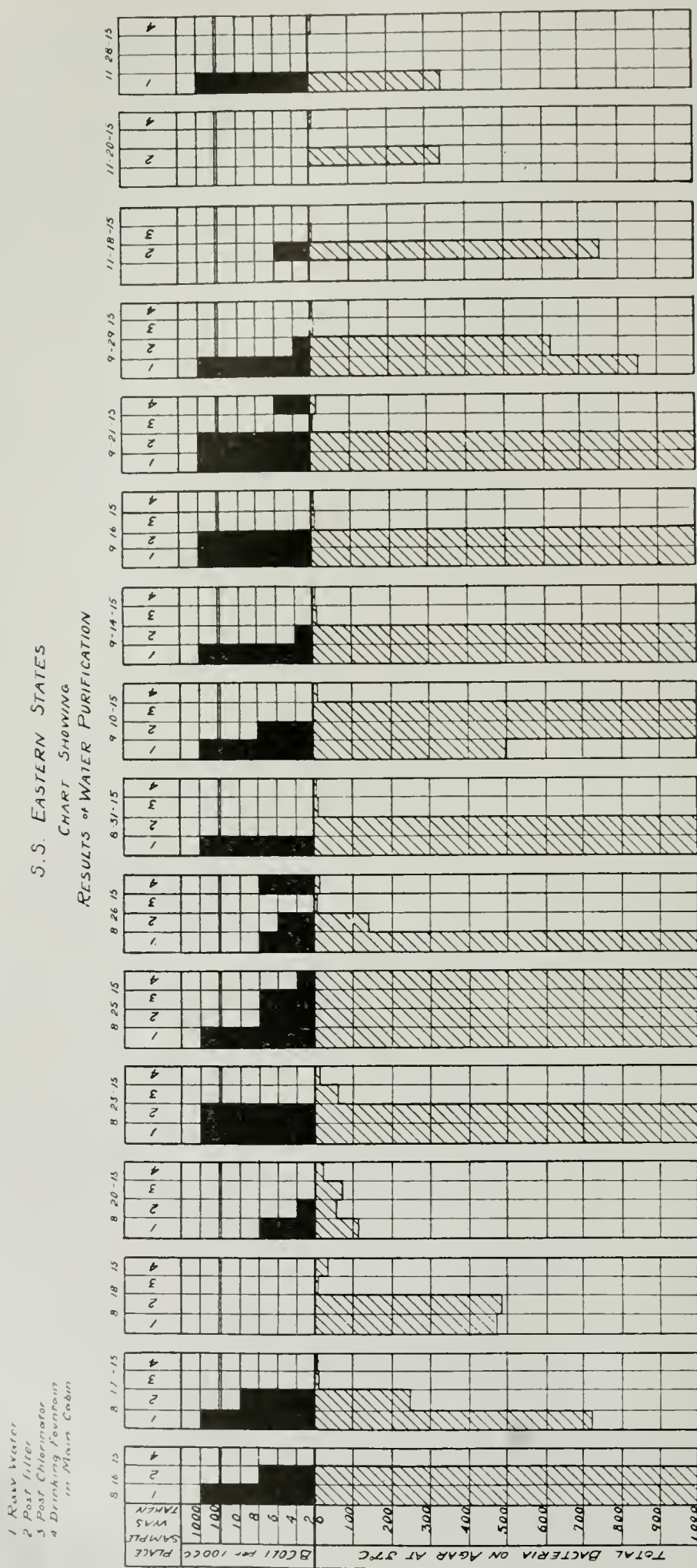


FIG. 24.

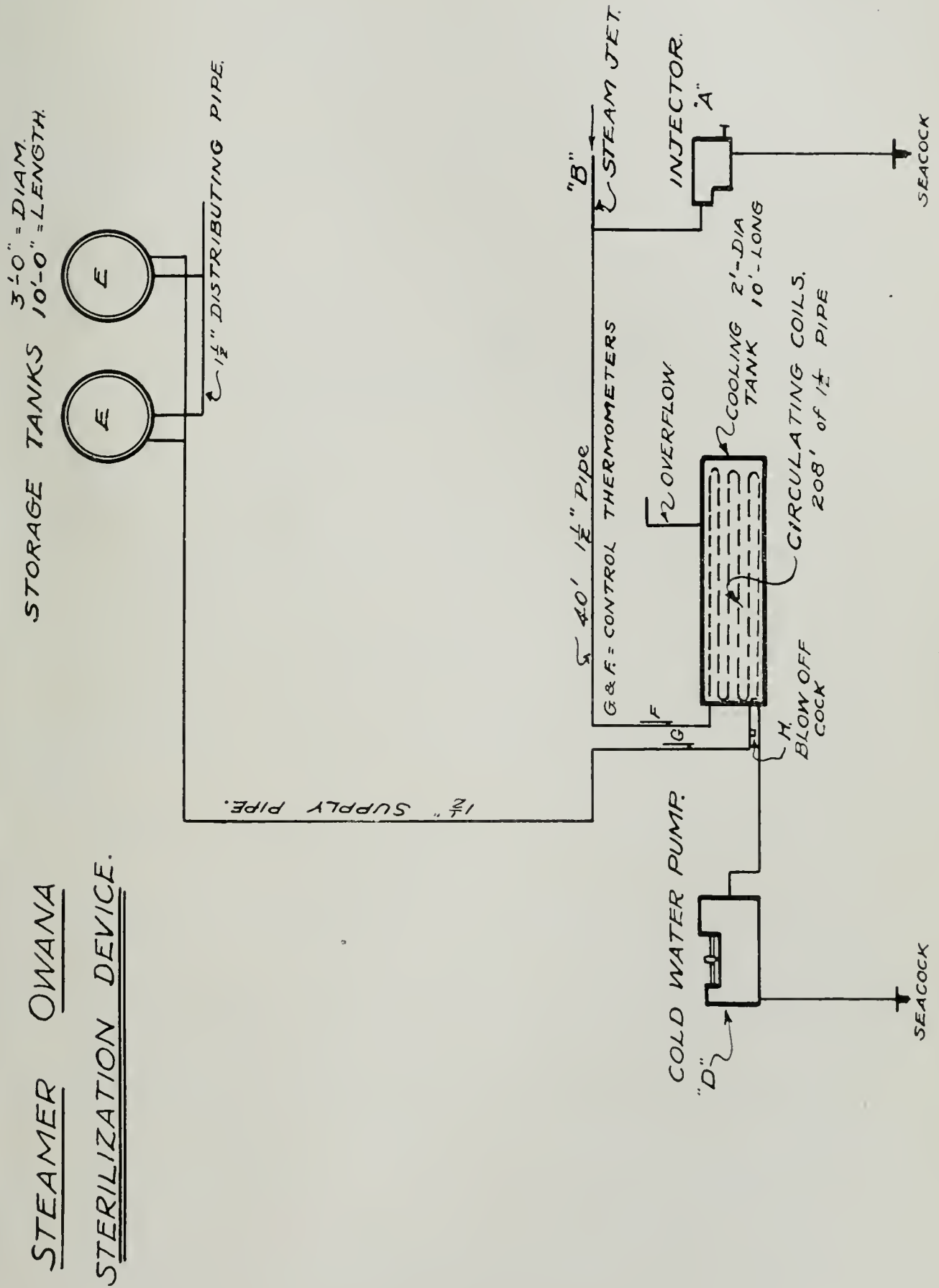
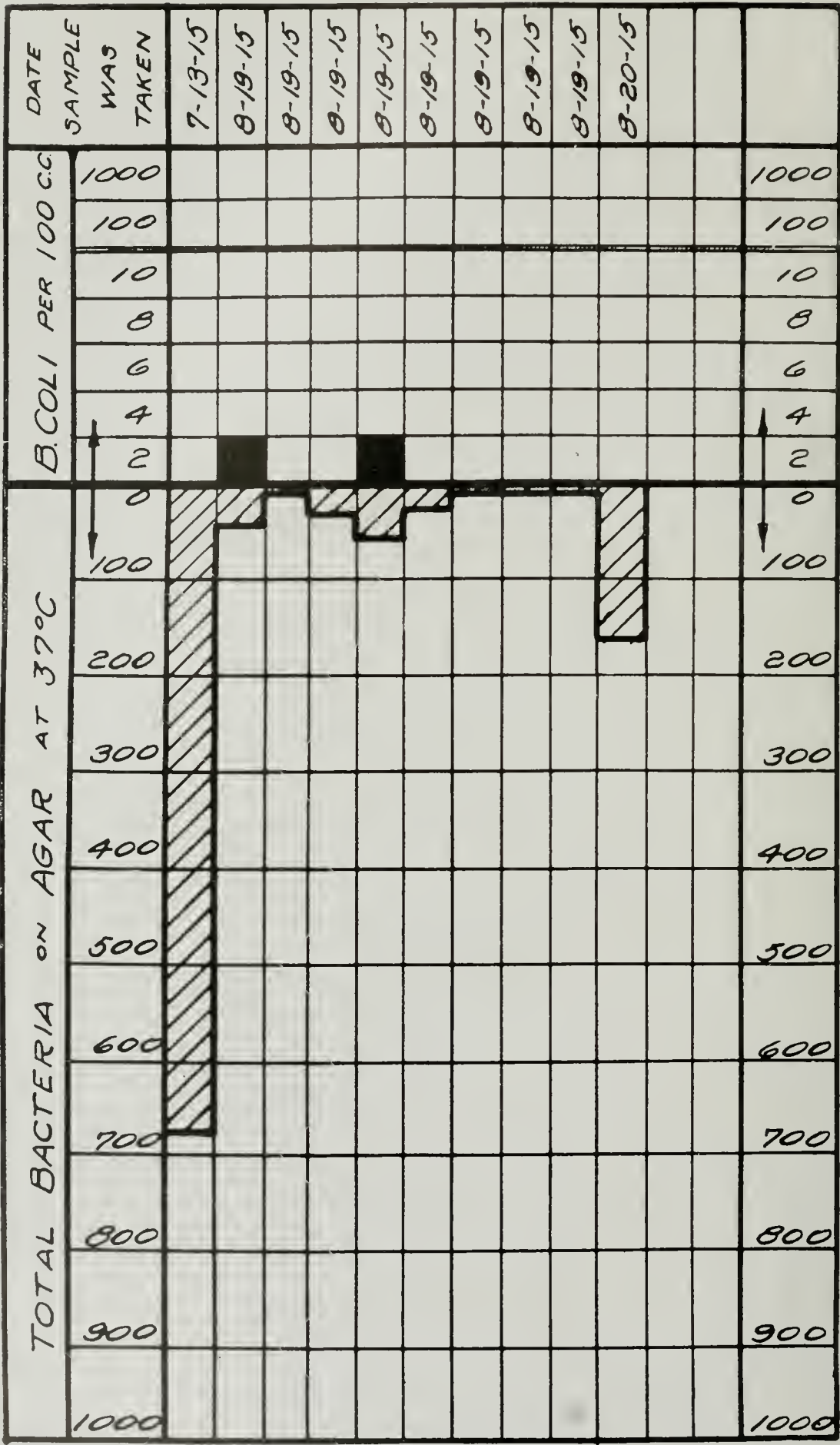


FIG. 25.



S.S. GREYHOUND

FIG. 26.

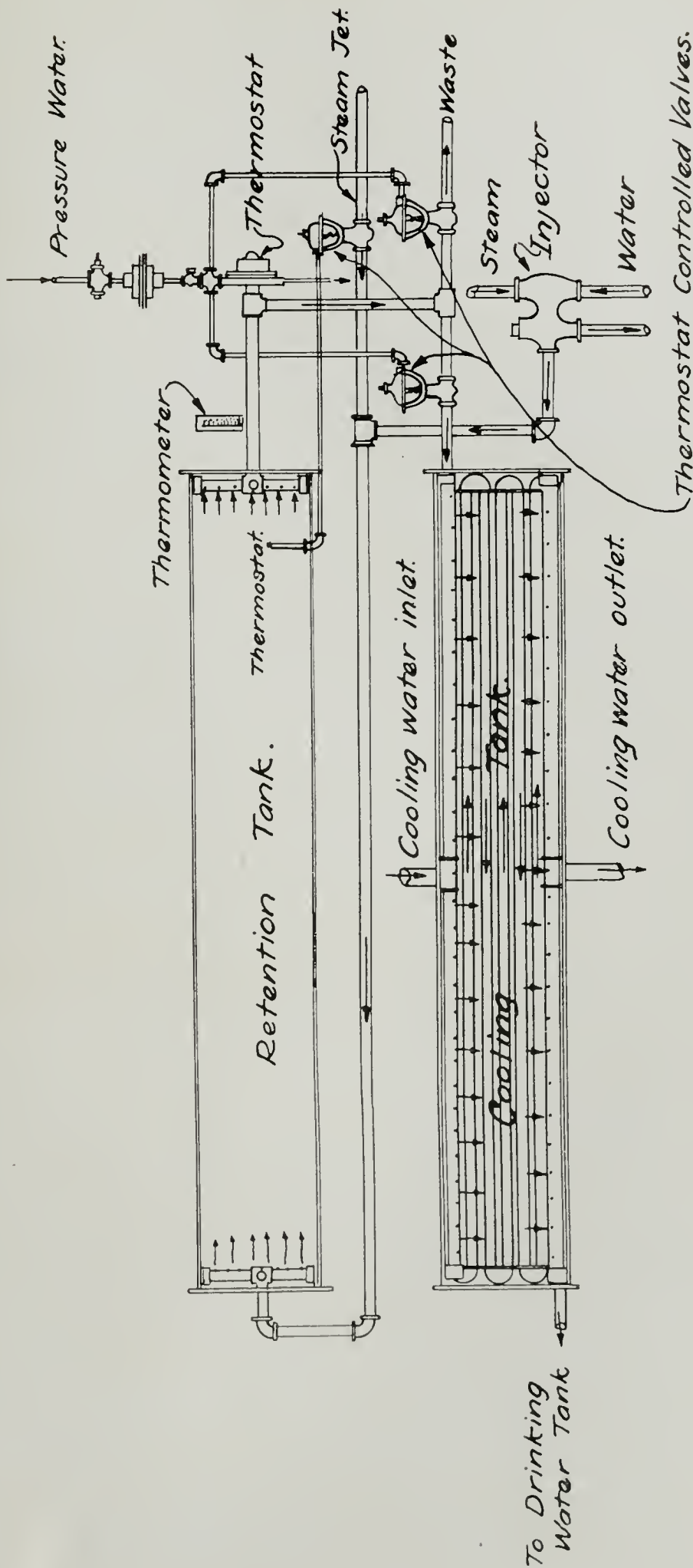


FIG. 27.

storage tank. The results of analysis of water treated by this method on a test run of the Steamer Greyhound are shown in Fig. 26.

The results of the first season's work have shown the need of devices for water purification which are automatic and which cannot in any way go wrong. Under the conditions that they must be operated, all of the apparatus must be "fool-proof" in order that at no time may the water be unsafe.

Because of the nature of the method, and the fluctuation in the demand, there seems to be at present no liquid chlorine apparatus which will meet this requirement.

The use of ultra-violet rays as a sterilizing agent was contemplated by one of the lines, but the idea was later abandoned for the following reasons:

1. The wide fluctuation in the voltage of the current on the boats causes the light to go out at each fluctuation and it does not light automatically, thus allowing water to pass unsterilized.

2. Necessity for filtration.

3. Danger of breaking candles, putting the apparatus out of order for an indefinite period.

An attempt has been made to obviate these difficulties in recent design, but it is still in the experimental stage.

Fig. 27 shows the changes made in the steam sterilizer which provide it with automatic control, so that no water may pass into the cooling tank which has not been heated to 220° for five minutes. It is thought that with these changes a nearly sterile effluent may be obtained. This improved device will be used by one line, at least, during the next season.

LABORATORY METHODS.*

The method of examination of drinking water in the laboratory is that adopted by the American Public Health Association at its last annual meeting. Briefly, it consists of the counting of plates inoculated with 1 c.c. portion of the water, one set being kept at 20° C. and counted after 48 hours; the other after 24 hours at 37°. Smith fermentation tubes are used for presumptive test for *B. Coli*. Five tubes are inoculated with 10 c.c. of the sample, and one tube with 1 c.c. These tubes are filled with lactose broth media and are incubated for 48 hours at 37°. If any show gas production during this time, portions are removed and streaked on freshly prepared Endo plates. If any of these streaks show a sheen of reduced fuchsin after incubation at 37°, a portion of the growth is inoculated into a second fermentation tube and incubated for 48 hours at 37°. Those tubes showing gas production and which are confirmed on Endo media and again show gas on incubation in lactose broth are counted as showing colon present. An enumeration of colon per 100 c.c. is made according to the Phelps Method. In addition 100 c.c. of water is mixed with concentrated lactose broth and incubated for 24 hours at 37°. Fermentation tubes are inoculated from this enrichment bottle and carried through the usual procedure as above. Fermentation, confirmation on Endo with gas production on refermenta-

*We are indebted to Mr. Roy W. Pryer, Director of the Board of Health Laboratory, for this data.

tion is taken to show the presence of 1 B. Coli per 100 c.c., providing the other fermentation tubes show no gas production.

ROUTINE MILK EXAMINATION.

The routine bacterial examination of milk is the enumeration of the number of organisms growing at 37° in 48 hours. Usually two dilutions are made; one, 1-1,000; the other, 1-10,000.

WIDALS.

Widals are made whenever physicians send in blood for this purpose.

Ordinarily, a few drops of blood are sent in a capillary tube. One part of serum is diluted to twenty parts with saline solution, and this solution is then added to an equal volume of typhoid suspension. Agglutination within one hour in a hanging drop is reported as a positive Widal. In many cases the test is made using paratyphoid a and b, as well as *Bacillus Typhosus*.

CONCLUSIONS AND RECOMMENDATIONS.

1. In view of all facts in connection with the situation it seems apparent that Detroit's typhoid during the past eighteen months has not been due to the unusual operation of a single factor but rather to the combination of several factors, each working independently of the other and one being more active at certain times than another.

2. It is evident that doctors have been somewhat at fault in their diagnosis. The laboratory department is now making every possible effort to cultivate the physicians and assist them by making Widals or other desired tests.

3. Milk infection has in the past been responsible for a marked percentage of typhoid cases but it is hoped that compulsory pasteurization will materially decrease the percentage of cases due to this source and it is thought that the compulsory physical examination of all employees who handle the milk after pasteurization would practically perfect the public protection.

4. Contact probably has played a more important part in the spread of typhoid infection in Detroit than any other single factor. The elimination of cases from this source of infection depends upon the widespread dissemination of knowledge concerning the cause and prevention of this disease and the strict enforcement of existing sanitary regulations.

5. Boats, freight and passenger, have added their share of cases. Too much emphasis cannot be laid upon the necessity of procuring and analyzing samples of water from each boat stopping at Detroit, the source and method of handling the water being equally important.

6. The presence of gross pollution along the river front is directly detrimental to the health of the citizens.

- (a) Possible pollution of municipal supply.
- (b) Pollution of factory supplies.
- (c) Pollution of bathing beaches and other means of direct contact with river water.
- (d) Cases of infection from river water imported from neighboring communities.

The elimination of this unwarranted river pollution should be effected by the adoption of proper sewerage and sewage treatment for the city and the compulsory sterilization of all sewage emanating from lake commerce.

7. Dr. Wm. H. Price in his annual report for 1914 states:

"Detroit may be said to have been more fortunate than otherwise in having limited typhoid to the present rate when all circumstances are considered. That the element of fortunate circumstances may not be overloaded, due attention should be directed toward purification of the present water supply."

The treatment of the municipal water supply by an oxidizing agent, such as hypochlorite of lime, liquid chlorine or a similar agent, should, at its best, be considered only as a temporary expedient. It is our opinion that in no case does a surface water which receives drainage from a territory occupied by human inhabitants or used for agricultural purposes, form a safe and satisfactory source of drinking water without some form of treatment or purification. Immediate steps should be taken to obtain for Detroit a water supply which will, without question, be clean and safe at all times.

8. The carrying out of the foregoing recommendations can probably best be brought about by the establishment of a sanitary district of sufficient size to include the drainage areas immediately affecting the local problems.

IMMUNITY IN TUBERCULOSIS

BY GERALD B. WEBB, M.D., COLORADO SPRINGS, COLO.

A COMBAT through almost countless generations with the tubercle bacillus has left most branches of the human race with a remarkable power of resistance to the parasite. We cannot claim, however, that we have any absolute immunity to the disease tuberculosis. On the contrary, tuberculin tests and postmortem records point to the probability of everyone having had at some time some degree of infection with this wax-coated bacillus.

NATURAL IMMUNITY.

We know of no animal race or human race which is not or cannot be infected with tubercle. Tuberculosis has been found occasionally in wild animals.—lions, tigers, panthers, jaguars.

Cattle would seem to have a relatively greater immunity to the bovine bacillus than man has to the human. In fact, Theobald Smith¹ has said that he believed cattle were nearly immune. This is borne out by statistical evidence. We find that practically each member of the human race is or has been infected with the tubercle bacillus, whereas from records kept at slaughter houses, the percentage of cattle infected varies from 15 to 35 per cent. The percentage of other animals found infected has been noted as follows: hogs 2.5 per cent, sheep and goats .1 per cent. The opportunity for infection must however be considered.

Gray mice are, it is thought, immune to both the human and bovine strains and only once was a softened focus found in experiments conducted by Theobald Smith.² Wells' experience corroborates this. The white or albino mice, however, can readily be inoculated with bovine tuberculosis, and field mice are very sensitive.

Spontaneous tuberculosis has been observed in carp, snakes, turtles and frogs. The bacillus affecting such cold-blooded animals will however not cause a general disease when inoculated into the warm-blooded species.

It is not yet settled that tubercle bacilli from warm-blooded animals can infect cold-blooded animals.

Parrots apparently can be infected with either avian, human, or bovine bacilli.

The hedgehog has been found to be a very resistant animal to infection.

Local lesions can of course be produced in any species by the inoculation of bacilli of other types. But such can be obtained by the dead as well as by the living, and Petruschky³ explains these lesions as being due to the irritating cell toxicity of the bacillary bodies.

According to Sofer,⁴ "The phthisical tendency is diminished for the northern race, just as the apoplectic tendency is for the Alpine, brachycephalic race."

No race of man which has had opportunity for infection has escaped. Tuberculosis of the spine has been observed in Egyptian mummies of the periods 3500 B. C., 2000 B. C., and 1500 B. C. In modern Egypt, however, tuberculosis was unknown. It has reappeared in Egypt and Palestine within the memory of physicians practicing there. This has been attributed to the introduction by infected Russian pilgrims, and to the return of native emigrants from America.

In recent years in Scotland and Ireland tuberculosis has spread from the towns to the country, and has become more widespread among the peasants which were formerly fairly free from the disease. As a result of modern rapid communication between various districts and countries, the disease may even become more widespread.

From an analysis of the death rate from tuberculosis, Karl Pearson⁵ has shown that, like other diseases, there would seem to be periods of increase and decrease in the prevalence of this disease. As Saundby⁶ states, we see this sequence in such diseases as plague, influenza, cholera, and typhoid fever; but the interval of time is short, lasting at most over a few decades. With tuberculosis, it apparently extends over centuries.

It appears that the present is to be considered a period of decline in the tuberculosis death rate, as in the last fifty years a decrease of almost 50 per cent has been observed both in England and America.

It was at one time thought that a certain definite immunity existed among the dwellers of high altitudes. It has been found, however, that this was due to lack of opportunity for infection.

Colorado Springs has a community largely composed of victims of pulmonary tuberculosis. Many of these have married usually healthy consorts, yet not infrequently have there been marriages between tuberculous victims. The writer has observed the children born to such marriages over a period of

twenty years. By means of von Pirquet tuberculin tests it has been found that all these children will react.

An occasional case of tuberculous meningitis and of an acute general tuberculosis has been observed, yet such are extremely rare. An outbreak also of open tuberculosis is exceedingly rare. The infection among these children is usually benign and easily overcome, and compares with that found in the slightly infected among healthy families in the lowlands.

Possible explanations for the disease being benign in this region are the large amount of open air life that is possible, and the increased numbers of lymphocytes the blood contains at such altitudes (Webb and Williams⁵³).

Certain families and individuals would seem to have a decided immunity to tuberculosis. Petruschky³ calls attention to what he designates "mother-immunity." It has frequently been observed that women originating from healthy families have married into a tuberculous family. They have not become infected by their tuberculous husbands, yet their children have one after the other died of tuberculosis. In the cases the writer has observed, the "mother-immunity" has been relative and not complete. Such mothers may react to the von Pirquet skin test, and one such was found to be herself a "carrier."

An inheritance of tubercle bacilli from infected parents is now regarded as being so rare a condition that we need not discuss it here. The so-called "habitus phthisicus" is today believed to be not a condition favorable to tuberculous infection, but a condition resulting from early tuberculous infection.

Neither is there an increased immunity enjoyed by the physically strong, such as athletes over the immunity of the frailer members of the race.

At one time it was considered that the children springing from tuberculous ancestors were less resistant than others, and marriage into such families was condemned.

The late Dr. Solly⁷ of Colorado Springs was one of the first (1895) to show that such offspring show more lasting cures and more chronic forms of pulmonary tuberculosis than those who had no tuberculous progenitors. Turban⁸ (1899) noted similar condition.

According to Reibmayer,⁹ the longer a region is pervaded with tuberculosis, the less becomes the death rate from tuberculosis in spite of the greater distribution of the infection.*

Petruschky believes that the large number of deaths in tuberculous families to be due to (1) the high virulence of the bacilli which have been tuned for that family, (2) the greater opportunity for massive infection, and (3) the earlier infection in childhood.

Children in their first year will almost invariably die from such infection. From year to year thereafter their resistance is increased, but by the school age greater opportunity for infection again takes place, and the mortality again increases. The next period of greater mortality comes at a time of life when the greatest efforts and strains of the bodily power are made and the resistance gained by a mild infection in childhood is over-ridden by a new infection from without or metastasis from within.

*Bushnell, Col. G. E.: Immunity Through Tuberculosis Infection (The Military Surgeon, 1913) contains an excellent review of this subject.

The question of racial immunity must be considered in relation to the opportunity for infection. In countries such as Turkey which was more or less unaffected, Deycke¹⁰ has shown that the importation of tuberculosis has been followed by a severer form and a more acute course of the disease.

Petruschky distinguishes several types of immunity in tuberculosis:

1. Genuine, persistent nonsensitiveness.
2. Different grades of relative resistance.
3. Cellular and humoral changes which can be brought about by immunization.
4. Defensive phenomena of the tuberculous organism.

The Bang system which has been tried in Denmark of breeding from tuberculin-reacting cows, would seem to be succeeding in producing healthy cattle for dairy purposes.

PATHOLOGICAL PROCESSES.

In the invasion of the body by the tubercle bacillus, the parasite strikes out at once for susceptible tissue. We do not find the first lesions in children in muscle tissue for instance, but in lymph nodes.

Ghon¹¹ believes there is always a primary lesion in the lung and from this the disease spreads to the nearest lymph nodes. Our own experiments with minimum lethal doses would seem to indicate that local lesions would depend on the resistance of the animal and also on the numbers and virulence of the bacilli injected. Natural infection most probably takes place with very few bacilli of varying virulence, and our experiments have indicated that it would be by no means unusual for an animal to escape a local lesion with such minute doses as ten to twenty bacilli.

Theobald Smith¹ states that it is the rule in bovine infection for the lymph nodes to become diseased without there being any lesion at the site of entry of the bacilli. Ravenel¹² showed that tubercle bacilli could pass through the normal intestinal wall. Smith has also pointed out that should bacilli escape from a primary focus in a lymph node, there is no miliary tuberculosis but isolated foci may appear in various organs. At times these foci may be absent and the infection of the organ only be indicated by disease of its corresponding lymph nodes. "In spontaneous infection the bacilli enter the lymphatics only, and the nodes act as a temporary or permanent barrier." In artificial subcutaneous inoculation, on the other hand, the bacilli may enter the blood direct.

From the lymph node infection the reactive processes cause a certain amount of general immunity as indicated by Koch's¹³ original experiments. Whereas the first infection may have left no local lesion at the point of infection, it is now quite different and as Smith says: "Clinically, the lymph node tuberculosis of children later becomes an organ tuberculosis, and pulmonary disease becomes the type of later life."

The production of organ tuberculosis would again depend on both numbers and virulence of the invading bacteria.

Tubercle bacilli probably pass through the alveoli of the lungs as soot does, possibly conveyed by phagocytes to the nearest lymph nodes which act

as filters. The bacilli finding a medium on which they can multiply through their ferments being able to split the tissues to suit their needs, the epithelioid cells respond by proliferation to defend the organism. This new tissue undergoes central necrosis and caseation, and the surrounding tissue proliferates and forms a dense capsule, so hedging around the bacilli. From the point of view of the parasite, this process is also a mechanism of defense, according to Smith.

In 1884 Metchnikoff¹⁴ pointed out that in the case of anthrax bacilli inoculated into the blood of lizards, animals which are in general very resistant to anthrax, these bacilli surrounded themselves with a transparent sheath placing themselves in a "state of defense." In a similar manner Metchnikoff found that the tubercle bacillus in the interior of the giant cells of the gerbil and under the noxious substances contained in these cells, envelopes itself in a transparent sheath. The action of the giant cell continuing, a series of such envelopes may be found. Theobald Smith^{1, 50} thinks that tubercle bacilli coming from a discharging focus are provided also with some more or less inert protecting substance as an envelope. This envelope has to be removed in some unknown manner both in the body and on culture media before the bacillus will multiply.

The poisoning effect of the tubercle bacillus is twofold. The bodies of the bacteria themselves can cause toxic processes, whilst the metabolism of the bacilli also produces a toxin. Dead bacilli inoculated under the skin can produce abscesses (Koch). The intravenous injections of dead bacilli into guinea-pigs and rabbits cause a wasting away and in time the death of the animals (Prudden and Hodenpyl¹⁵). The fever production in tuberculosis is however due to the metabolic products of the bacilli, and not to the bacilli themselves (Cornet and Meyer¹⁶).

Aberrant forms of tuberculosis are most common in childhood. Such are infections of lymph nodes, meninges, bones, joints, etc. Theobald Smith thinks this is because the tubercle bacillus is keyed to adult life and chiefly to the lungs where as a true parasite it can have access and egress.

TISSUE SELECTION.

The tubercle bacillus has been found in practically all the tissues of man, yet infection of muscle tissue and brain substance is indeed rare. Councilman's¹⁷ description of tuberculous brain tumors is worth quoting: "Elsewhere in the body tuberculosis, save in the unusual types of the disease in the lymph nodes, but rarely appears in tumor form. In the cerebral tissue the disease takes the form of distinct circumscribed nodules which may reach the size of a walnut; they are multiple or single, and have little tendency to central softening. They present such differences from tuberculosis elsewhere that before the identity of the disease was established they were given a special designation. They are commonly associated with a chronic form of meningeal tuberculosis which may be the primary or secondary infection. Tuberculosis of the lungs in some degree is always present. The disease is twice as common in the cerebellum as in the brain. The solitary tubercles take the form of hard white masses, round or irregular, and surrounded by a zone of more active

tissue in which miliary points of caseation can be distinguished. The nodule increases in size by the formation of miliary foci immediately around it which becomes included in the central caseous mass."

There can be no doubt that the tubercle bacillus is especially suited to attack the lymph nodes of children and the lung tissue of adults. Should other tissues be invaded, it may be partly the result of accident and partly due to variations in the bacillus or the soil.

THE RELATION OF THE DUCTLESS GLANDS.

The most important recent work in connecting a ductless gland with tuberculosis immunity is that of Lewis and Margot.¹⁸ They observed that in mice and rats infected with virulent cultures of bovine tubercle bacilli an almost constant hyperplasia of the spleen was noticed. Lewis and Margot found that removal of the spleen in albino mice greatly increased their resistance to an intraperitoneal infection and limited the generalization of the infection. That such a result was not due to interference with the distribution of the bacilli through the portal circulation was proved by subsequent experiments. In these it was found that splenectomized mice which had become tolerant to the feeding of splenic tissue showed no more resistance to tuberculous infection than normal mice. Lewis and Margot conclude therefore that when a spleen is removed some bodily function which makes for susceptibility to infection with the tubercle bacillus is also removed.

An enlargement of the thyroid gland is very frequent in early cases of pulmonary tuberculosis, whereas in late stages of the disease this gland would appear to share in the general atrophy. When enlarged, it is usually soft to the touch.

A small number of experiments were undertaken by Dr. Gilbert and myself to study the effect of the removal of the thyroids upon tuberculosis in guinea-pigs. Portions of the thyroid gland varying from three-fourths to the whole amount were removed under ether. Under similar conditions, the thyroids of the control pigs were exposed but not removed. Four weeks later each pig received in the nipple area a subcutaneous inoculation of forty living virulent human tubercle bacilli. The general results may be briefly summarized as follows:

Series A.—Three-quarters of the thyroid tissue was removed.

1. The general condition and appearance when killed was strikingly different. While the control pigs were thin and emaciated and dying, the others were fat and healthy in appearance.
2. The amount of tuberculosis produced did not differ materially in the two sets of pigs.
3. Local lesions were more frequent and prominent in the controls.
4. Hyperemia of the remains of thyroid tissue was noted at autopsy.

Series B and C.—All thyroid tissue was removed.

1. The extent and distribution of the tuberculosis were not affected.
2. The pigs without thyroids showed marked signs of myxedema.

In general, tuberculous guinea-pigs which have been killed during the course of the disease have shown enlargement of the thyroid gland.

The influence on the course of established tuberculosis was also studied. The tuberculous pigs from whom the thyroids were removed died earlier and had more extensive tuberculosis than the controls.

These experiments would seem to suggest that in tuberculous infection in guinea-pigs the thyroid gland tends to hypertrophy but that any decided bearing on immunity processes is not detected.

In chickens infected with the "*Spirocheta Gallinarum*" enlargement of the spleen and also of the thyroid gland has been noted. Launoy and Bruhl¹⁹ experimented with this disease after the removal of each of these glands and they were unable to determine that their removal increased the resistance of the animals. Removal of the thyroid did not modify the infection. Removal of the spleen appeared to increase the rapidity of the disease.

Clinicians have noted the increase of temperature at the menstrual periods of tuberculous women. The effect of tuberculosis on menstruation can be quite marked, so that upwards of 50 per cent of cases may suffer from some degree of amenorrhea. In advanced cases many tuberculous women cease to menstruate, and this has been noted also in the earlier stages, especially in young girls. We see not uncommonly tuberculous women having hemoptysis at every menstrual period for several consecutive months. Macht²⁰ in summarizing the influence of menstruation on a tuberculous process states:

1. There can be an aggravation of all symptoms and accentuation of physical signs.
2. The effects of "ovulation" may continue after the menstrual flow has been suppressed.
3. Periodic variations in temperature are very common and occur in probably 50 per cent of all cases.

Hollos²¹ believes that such toxic phenomena in tuberculous women stand in some relation to the degree of immunity possessed by the individual.

THE RELATION OF THE BLOOD.

Bartel²² noted a defensive action of the lymphocytes to tuberculous infection. It has been long recognized that lymphocyte cells take a large share in the formation of tubercles. Bergel,²³ also Marie and Fiessinger²⁴ were able to prove that lymphocyte cells contained a lipase which could split the wax of tubercle bacilli. Tubercle bacilli themselves have also been found to contain a lipase. Such a quality may help to explain the selection of tubercle bacilli for lymph nodes. It would seem possibly a paradox that lymphoid tissue should exhibit a definite role and yet be the choice selection for these bacilli.

Murphy²⁵ showed in animals whose lymphoid tissue had been destroyed by x-rays a greater susceptibility to tubercle bacilli.

Children have a larger number of lymphocytes (6,000 per cm.) in their blood than adults, and this increased number diminishes at puberty to the adult picture (3,000 per cm.).

For a long time it has been noted that the blood platelets were usually in-

creased in all forms of tuberculosis. Nature rarely increases such elements without a definite purpose. Leucocytosis in pneumonia and in other affections is better understood since the phagocytosis discoveries of Metchnikoff.²⁶

We²⁷ have reported experiments which would indicate that blood platelets either carry or supply opsonins. Duke²⁸ has shown that these elements are decreased by large injections into guinea-pigs and rabbits of different bacterial elements but that small doses stimulate an increased production of platelets. We have found in a few cases of miliary tuberculosis a great diminution of platelets, but in almost all cases of pulmonary tuberculosis and in the tuberculosis of laboratory animals we have noted an increase of these elements.

ARTIFICIAL IMMUNIZATION AGAINST TUBERCULOSIS.*

Almost the greatest prize in preventive medicine today is to find a method of immunizing the human race against tuberculosis. With hygiene alone small-pox cannot be controlled, and it is safe to say that hygiene will never be sufficient alone to prevent tuberculosis. America has always been in the front ranks in endeavoring to discover a method of immunization. I would refer the reader to a review of these efforts in a paper by the author²⁹ in 1908. The foreign work is reviewed well by Cornet and Meyer.¹⁶

We must remember at the start, that in successful vaccination of all diseases known today, absorption of the virus by phagocytosis is the essential condition of success. The tubercle bacillus does not lend itself to such absorption.

In any discussion of artificial immunization we must always recall the early experiments of Koch.¹³

"If a normal guinea-pig is inoculated with tubercle bacilli, the point of inoculation very soon closes. After ten to fourteen days there appears at this site a small hard nodule which finally ulcerates. This shows no tendency to heal, and remains so until the death of the animal. If, however, an already tuberculous guinea-pig is similarly inoculated, while the point of inoculation also closes, no indurated nodule appears. Instead, a necrotic process of the skin sets in after the second day, which finally terminates in the casting off of the slough and the formation of a flat ulceration that heals rapidly. Neither do the neighboring lymph nodes become infected. It does not matter whether living or dead tubercle bacilli are used for the second injection."

Romer³⁰ found that tubercle bacilli injected into a tuberculous animal might not be destroyed at the site of inoculation but could be held in the tissues in a living and virulent state without the production of a tuberculous lesion, and even multiplication might take place. He failed to demonstrate a lytic process such as can be observed in the Pfeifer phenomenon for instance.

Manwaring and Bronfenbrenner³¹ claim to have demonstrated a lytic process brought about by the peritoneal cells in tuberculous guinea-pigs. They proved that such power was not connected with any substance present in the circulating blood.

*Since this was written a paper has appeared in the *Journal of Out-door Life* by Dr. Victor G. Heiser, in which it is claimed that tuberculosis has been practically eradicated from the State of Victoria. Further confirmation of this will be needed, yet the methods employed seem well worthy of being copied.

Kraus and Hofer³² found that tubercle bacilli injected into the peritoneal cavity of tuberculous guinea-pigs were destroyed quickly by lysis.

Hamburger³³ also demonstrated by experiment that all the bacilli of the second inoculation are not always killed. He found that some months after the second inoculation, a tuberculous process may appear and develop like a primary infection.

Romer³⁰ has shown that Koch's original experiments only hold good provided:

1. The first infection must be a weak one allowing the disease to run a chronic course. (Koch's guinea-pigs had been infected some weeks when he gave the second inoculation.)

2. The intervals between the first and second inoculations must be long enough to have allowed the development of some degree of immunity.

3. The number and virulence of the second inoculation must not be too great.

Vaughan's³⁴ explanation of the changed reaction to Koch's second inoculation is that the lytic agent which destroys the tubercle bacillus is produced in large amount in tuberculous animals because the cells of these animals have been sensitized and store up a zymogen which is activated when tuberculo-protein is brought in contact with the cell. "The ferment is capable of destroying only a given amount of bacilli or is wholly inactive in the presence of a great amount of substrate, or its action is soon interrupted by the accumulation of fermentative products."

It may be that while some bacilli can be destroyed others have time to develop their protecting sheath and so defy destruction.

In Koch's experiments, it is probable that these bacilli were largely thrown off in the slough, many possibly unchanged.

Koch's conclusions from all his work in the production of immunity to tuberculosis lead him to state that he believed tubercle bacilli could never be absorbed living or dead, and at the same time he would never expect any appreciable immunity to be produced except by the use of living and virulent bacilli.

Calmette³⁵ is of the opinion that tubercle bacilli are never destroyed by the body cells and is certain they can be eliminated by the bile and at times by the kidneys.

Pathologists agree that they rarely if ever have seen tubercle bacilli undergoing lysis.

Much³⁶ has claimed that a type of the bacillus free of the waxy covering similar to that sometimes found in early cultures can be discovered in tuberculous lesions and in tuberculous discharges. Much's hypothesis, however, is not yet firmly established and cannot yet be offered in explanation of the disappearance of tubercle bacilli in lytic experiments such as those conducted by Manwaring.³⁷

Löwenstein³⁸ has said that "only the tuberculous organism is tuberculosis immune."

Vaughan has found the tubercle protein to contain less poison than the

cellular protein of the colon and typhoid bacilli. By means of splitting up the cellular substance of tubercle bacilli with alkali in absolute alcohol, Vaughan has obtained a poison similar to bodies which have been procured from bacterial vegetable and animal proteins. Given in sufficient quantities, the poison kills within an hour both healthy and tuberculous animals. Vaughan concludes from his work that no preparation from the tubercle bacilli should be used in the treatment of tuberculosis until the poisonous group of the tuberculous protein be removed.

Obtaining the cellular substance from tubercle bacilli by extracting first with alcohol and then with ether, Vaughan has found that with it guinea-pigs may be sensitized to the tubercle bacillus.

Baldwin³⁹ and later Krause⁴⁰ have made important contributions to our knowledge on sensitization to tubercle protein but have discovered no new procedure so far in the production of appreciable immunity to tuberculosis.

Krause's conclusions are as follows:

1. Sensitization of nontuberculous guinea-pigs with tubercle protein does not alter their resistance to experimental tuberculous infection.

2. Sensitization to tubercle protein and relative immunity (increased resistance) to infection can occur coincidentally in the same animals.

Besredka⁴¹ believes that of all varieties of vaccines, sensitized living vaccine is able to develop the maximum amount of protective substances. To prove that the use of such is without danger in certain diseases, Besredka shows that 15,000 people have been inoculated with a vaccine prepared from living typhoid bacilli without local or general reaction resulting. In addition, hundreds have been treated with living sensitized staphylococci, streptococci, gonococci, etc.

In applying such principles to immunization in tuberculosis, Baldwin⁴² has found that by the addition of an immune serum obtained from a cow to living tubercle bacilli, animals became infected more severely than the controls.

We⁴³ have inoculated guinea-pigs with a minimum lethal dose—about thirty bacilli—after incubation with immune serum obtained from tuberculous guinea-pigs. The resulting infection has been similar to that of the controls.

We⁴⁴ have also added to such minimum lethal doses of virulent tubercle bacilli "immune" platelets and "immune" lymphocytes obtained from tuberculous guinea-pigs, and we have found that infection is not constantly avoided and also that the infection resulting has been similar in degree to that of the controls. We do not believe, therefore, that there is any way of sensitizing virulent living tubercle bacilli to render them of service for vaccination.

It would now seem to be quite settled that the inoculation of human bacilli into cattle for immunization purposes has proved a failure, both because of the short duration of the immunity and the dangers from the bacilli getting into the milk.

The partial immunity of animals inoculated with bacilli of low virulence such as in Trudeau's⁴⁵ experiments, has possibly always been due to the production of mild unrecognized infections in these animals.

We know today that the human race is constantly exposed to infection by tubercle bacilli, the numbers and virulence of which are matters of chance to

each individual. From such chance infections, however, the race becomes in a crude manner vaccinated to a certain degree against the disease. Nature, however, in doing so, causes the death of many individuals, especially in childhood.

The consensus of opinion among those who have tried to produce immunity in animals is that Koch was correct in recognizing that the living bacillus must be employed. Unlike the successful immunization against typhoid with dead bacilli, in tuberculosis it would appear to be necessary that there should be a struggle between the living bacillus and the living cell before any appreciable immunity is registered. Such a contest is probably one of ferment action.

This was indeed the basis of the original hypothesis of Welch,⁴⁶ that "in the struggle between the bacteria and the body cells . . . each participant is stimulated by its opponent in the production of cyto-toxins hostile to each other, and thereby endeavors to make itself immune against its antagonist."

Besredka⁴¹ has shown that in general animals immunized with living sensitized organisms develop some agglutinating power, but their serum has a high bactericidal power and is rich in antibodies. Animals, on the other hand, immunized with heat-killed organisms develop a marked agglutinating power, but their serum is weak in bactericidal powers and the amount of antibodies is negligible.

In tuberculosis, the protection due to antibodies would seem to be of secondary importance to that due to the lytic powers of the fixed tissue cells.

Antibodies such as specific lysins are not easily demonstrated in the blood of tuberculous animals or people.

The parenteral introduction of foreign proteins in general is followed by the production of specific antibodies. Such specific antibodies have not yet been shown to be of the nature of specific protective ferments. The work of Opie⁴⁷ and later Manwaring suggest, however, that the tubercle bacillus is opposed by cell ferments.

The lack of very demonstrable specific antibodies in the serum of tuberculous animals would presuppose the failure that has been met in attempts to develop a passive form of immunity. On the other hand, by the use of a special growth of tubercle bacilli as an antigen, Besredka⁴⁸ has found that it would seem possible to use the methods of complement fixation to recognize the presence of an active tuberculosis.

With the knowledge we now have that a very few bacilli can produce tuberculosis, it would seem wise to experiment more with definite numbers rather than with moderate quantities of bacilli.

It is realized that practically the whole human race is infected at some time with this disease, and that the infection takes place with bacilli whose numbers and virulence are beyond our control.

The ingenious technic elaborated by Barber⁴⁹ for the isolation of single organisms allows us to produce infection of guinea-pigs with a small definite number of tubercle bacilli.

It frequently happens that when a small lethal dose—say twenty virulent tubercle bacilli—is inoculated subcutaneously, no local lesion will be produced.

In such cases, the nearest lymph nodes usually first indicate disease. Similarly the inoculation of a small lethal dose into an already tuberculous pig is never followed by a necrotic process in the skin.

The local lesions of Koch's original experiments hold good only then for the inoculation of quantities or moderate quantities of bacilli, and not for minimal quantities. The processes of immunity, however, indicated by the changed reaction to the second inoculation hold good for all time and are the foundation on which almost all subsequent research has been laid.

LOCAL IMMUNITY.

Smith¹ found that by injecting a suspension of dead tubercle bacilli into the peritoneal cavity of a guinea-pig, in a few weeks the animal became sensitive to tuberculin. Should a second similar dose be injected then into the peritoneal cavity, there is a tuberculin reaction accompanied by fever and loss of weight. This reaction Smith thought to be due to the rousing of a local immunity by his first reaction. Inoculating similar guinea-pigs under the skin, he found there was a slight degree of general immunity, but this was distinctly below the influence imparted locally. Smith advocates the trial of whole bacilli killed by heat at 60° for immunization of the human race, stating that such bacilli are much more efficacious than old tuberculin.

The very objection that Koch found in the bodies of the bacilli forming an abscess Smith feels would be advantageous by causing a local focus as in the subcutis from which immunity might radiate.

CLINICAL CONSIDERATIONS.

The altered reactivity toward tubercle bacilli in an infected person is spoken of as allergy. This allergy is due to the infecting organism possessing antibodies which can supposedly digest the bacillus and its poisons.

A result, possibly comparable to Koch's second inoculation, is evidenced when an allergic individual is subjected to a new lung infection. A cavity is formed, a result similar to the necrosis and sloughing of the skin as described by Koch, but in this case, healing is not so certain.

A patient with measles is particularly vulnerable to many infections, such as diphtheria, influenza, and especially tuberculosis. Should measles occur in a tuberculous individual, a condition of anergy is produced, and the reaction to tuberculin is lost.

In childhood the condition recognized as scrofula on the other hand brings with it a condition of hyperergy or exaggerated activity. Such scrofulous children usually get well, and later in life would seem to have a higher immunity against pulmonary disease than their fellows.

Baldwin² has shown that by the removal of tubercles all sensitiveness to tuberculin disappears, and soon all immunity to the disease is lost. This observer claims that the same thing happens when tubercles become thoroughly fibrous or calcified, and he concludes that in the allergic condition of the tuberculous is contained the relative immunity. It would appear therefore that from

a tuberculous focus a constant stream of some sensitizing material emanates which keeps tuned up the cells of the distant tissues.

When patients are kept under sanatorium regime they regain their tolerance for the bacillus, and the majority of open cases become "carriers." By prolonged rest these people are freed to a very great degree from massive re-infective metastatic doses of the bacilli and are able to withstand small dosage. The regime of sanatorium treatment reduces the allergic condition of the individual, the aim being to promote encapsulation and fibrosis. Clinicians frequently have noted the improvement in health in those cases which have developed a serous pleural effusion. We are observing this possibly more today than formerly, owing to the usage of artificial pneumothorax. From such serous fluids we have readily grown tubercle bacilli.

Baldwin⁵¹ has noticed benefit in such cases when the fluid has been decidedly purulent, and we have also observed similar conditions.

T. von Mural⁵² claims that in pleural exudates there is a decided beneficial effect through absorption of the theoretical antibodies contained in the exudate.

In the human race as age advances, after forty, the death rate from tuberculosis decreases. Elderly people also do not react to tuberculin as do young people, and yet they would appear to have a high degree of resistance to tuberculosis.

IMMUNIZATION BY THE INOCULATION OF LIVING ORGANISMS BEGINNING WITH ONE OR A FEW ORGANISMS.

Koch decided that a living and virulent bacillus must be employed to produce successful immunity against tuberculosis. Koch also decided that "we shall not succeed in habituating the organism to absorbing entire bacilli which have been injected subcutaneously; and by injecting small quantities of them, we shall not habituate the organism to absorbing more."

For several years we have been engaged in attempting therefore the seemingly impossible. With a human culture several years old, we were enabled to inoculate guinea-pigs successfully with gradually increased numbers, which totaled many thousand times the lethal dose⁵⁴ (M. L. D. 125 bacilli). With recently isolated cultures (M. L. D. 10 bacilli), we have always failed in this method.

Inoculating a series of monkeys⁵⁵ (*Macacus Rhesus*) with gradually increasing numbers of a human culture of which the minimum lethal dose was thirty-five bacilli, we were successful in some monkeys in inoculating totals of over ten thousand times this lethal dose. The examination of the tissues and the injection of different macerated lymph nodes and organs into guinea-pigs failed to show any tuberculosis. We therefore have felt that a true immunity may be produced in this manner.

Unfortunately all monkeys were not so successfully inoculated, and one became infected with even small doses cautiously increased, and before it had reached 1,000 bacilli. Out of a series of six monkeys, four were successfully inoculated and two failed. One of the failures has just been referred to, and in the other case the dosage was increased unusually rapidly. With a culture

somewhat less virulent, we should expect constant success in monkeys, judging by our experiences with guinea-pigs.

The question at once arises, will the modified virulence remain so or will the bacilli after inoculation become more virulent. This question only a larger number of experiments can determine.

Having isolated many cultures from different cases of pulmonary tuberculosis, we have found some variance in their virulence judged by the smallest numbers required to kill guinea-pigs.

The children inoculated five years ago with a culture of which the minimum lethal dose for a guinea-pig was found to be 125 bacilli still remain in perfect health and do not respond to the von Pirquet test. The method is unfortunately not practical and yet further experimentation might make it so.

From our work we have been convinced that infection takes place usually in childhood with very small numbers of bacilli, and that if an immunity in childhood can be raised to even a slight degree we should probably achieve a successful vaccination to tuberculosis.

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TISSUE CELLULAR PROTEIN POISONS*

BY JAMES G. CUMMING, M.D., BERKELEY, CALIF., AND JOHN S. CHAMBERS, M.S.,
 ANN ARBOR, MICH.

VAUGHAN'S protein poison can be derived not only from pathogenic bacteria, but also from nonpathogenic, from proteins of the vegetable world, from albumins or globulins, and finally, as shown in this paper, from the tissue cells of exsanguinated organs of multicellular organisms.

Since as Vaughan has pointed out, although bacteria are usually classified as microscopic plants, yet owing to their chemical constitution they properly belong to the animal kingdom, it was assumed in the work here reported, that the protein poison could be obtained from the tissues from multicellular as well as unicellular animals.

Tissues including muscle, brain, heart, lungs, liver, pancreas, and kidney were obtained from the dog, goat, ox, and rabbit. Because the protein poison can be obtained from blood constituents it was necessary to remove these before clotting occurred, thus securing organs free from water soluble protein. The following procedure was carried out to gain this result with the dog, goat, and rabbit; the animal was first anesthetized, then a glass cannula was introduced into the abdominal aorta; the jugulars were then cut and water was forced under mild pressure into the circulatory system. Several liters of wash water were used in each case, the washing being continued until the fluid running from the jugulars had lost its red tinge. The ox organs were removed immediately after death, and each was washed free from blood by forcing water through its circulatory system. The organs were cut into half-inch cubes and washed in water with frequent changes; the final wash water gave none of the tests for protein. The tissues were then dehydrated in 80 per cent and 100 per cent alcohol each for twenty-four hours. They were then dried and powdered with a fine meat grinder.

Vaughan's standard procedure for splitting off the poison is here quoted:

*From the Hygienic Laboratory of the University of Michigan.

The powdered tissue was extracted in a Soxhlet apparatus, first with alcohol until the wash alcohol was colorless, then with ether for from twelve to eighteen hours. The pure tissue protein thus obtained was dried and then split into its poisonous and non-poisonous parts with an alcoholic solution of 2 per cent sodium hydrate in the proportion of 250 c.c. of the alcoholic solution to 10 grams of the pure protein. The splitting process was continued for an hour at 78° C., the alcoholic mixture being kept agitated during this time by a mechanical mixer. The insoluble nonpoisonous part was then separated from the soluble poisonous part by filtration. The sodium hydrate was neutralized with hydrochloric acid, and the alcoholic insoluble sodium chloride was filtered off. The alcoholic solution of poison was then evaporated to dryness, taken up again in alcohol to remove the last trace of salt, and finally evaporated. The poisonous residue—the final product—was then powdered.

Tabulation A gives the loss in weight as a result of dehydration and drying, extraction with alcohol and ether, and the final percentage yield of the split poisonous part.

TABULATION A.

SOURCE.	FRESH TISSUE.	DRY SUBSTANCE.	AFTER EXTRAC- TION WITH ALCOHOL AND ETHER.	PERCENTAGE YIELD OF POISON.
Ox liver	235 gms.	49 gms.	44 gms.	28*
Ox heart	245 "	57 "	37 "	37
Ox kidney	250 "	37 "	33 "	32
**Ox blood cells	2,500 c.c.	2.8 "	2.6 "	25
Dog muscle	320 gms.	82 "	52 "	66
Dog liver	255 "	56 "	43 "	46
Dog spleen	32 "	7 "	5.5 "	36
Dog kidney	52 "	11 "	10 "	31
Dog pancreas	31 "	4.3 "	3.8 "	32

*This is the percentage yield of poison obtained from the protein after extraction with alcohol and ether.

**The ox blood cells were prepared by first drawing the fresh blood into sodium oxalate solution to prevent coagulation. The blood cells were then washed repeatedly with water, their separation being facilitated by centrifugation after each washing. The washing procedure was continued until the cells were free from hemoglobin and serum. So here we have cells which are free from water soluble protein, thus, only that part of the cell remains which enters into the actual makeup of the cell-ground substance.

Tabulation B gives the sources of the poisons, their doses intraperitoneally, and the results in guinea-pigs.

TABULATION B.

SOURCE OF POISON.	INJECTION INTRAPERI- TONEALLY.	RESULTS IN GUINEA-PIGS.
Ox liver	25 mgs.	Severe symptoms.
	50 "	Peripheral irritation, paralysis, death 12 minutes.
	100 "	Convulsions, death 10 minutes.
Ox heart	25 "	Mild symptoms.
	50 "	Convulsions, death 20 minutes.
	100 "	Peripheral irritation, convulsions, death 23 minutes.
Ox kidney	25 "	Severe symptoms.
	50 "	Per. irr., death 6 minutes.
	100 "	Conv., " " "
Ox blood cells	25 "	Severe symptoms.
	50 "	Per. irr., par. paral., death 17 minutes.
	100 "	Ruf. coat, per. irr., par. paral., death 20 minutes.

TABULATION B.—Continued.

SOURCE OF POISON.	INJECTION INTRAPERITONEALLY.	RESULTS IN GUINEA-PIGS
Dog muscle	25 mgs.	Mild symptoms.
	50 "	Severe symptoms.
	100 "	Par. paral., conv., death 20 minutes.
Dog liver	25 "	Per. irr., par. paral.
	50 "	" " " "
	100 "	Conv., death 12 minutes.
Dog spleen	25 "	Conv., death 20 minutes.
	50 "	Par. paral., lab. resp., death 20 minutes.
	100 "	Conv., death 15 minutes.
Dog kidney	25 "	Mild symptoms.
	50 "	Severe symptoms.
	100 "	Per. irr., par. paral., death 10 minutes.
Dog pancreas	25 "	Quite marked symptoms.
	50 "	Mild symptoms.
	100 "	Per. irr., conv., death 6 minutes.
Dog heart	25 "	Per. irr., ruf. coat, par. paral., conv., death 12 minutes.
	50 "	" " conv., death 18 minutes.
	100 "	Par. paral., death 15 minutes.
Dog lung	25 "	Par. paral., conv.
	50 "	Lab. resp., conv. death 11 minutes.
	100 "	Per. irr., par. paral., death 8 minutes.
Goat muscle	25 "	Ruf. coat, conv., death 20 minutes.
	50 "	Per. irr., conv., death 14 minutes.
	100 "	Par. paral., death 5 minutes.
Goat kidney	25 "	Per. irr.
	50 "	Par. paral., conv., death 17 minutes.
	100 "	Par. paral., ruf. coat, recovery.
Goat spleen	25 "	Par. paral.
	50 "	Lab. resp., par. paral., conv., death 12 minutes.
	100 "	Per. irr., conv., death 8 minutes.
Goat heart	25 "	Per. irr., ruf. coat, par. paral.
	50 "	Par. paral., death 30 minutes.
	100 "	Per. irr., par. paral., conv., death 12 minutes.
Goat brain	20 "	Par. paral., ruf. coat, per. irr.
	30 "	" " " "
	50 "	Ruf. coat, lab. resp., par. paral., conv., death 20 minutes.
Goat lung	25 "	Par. paral., lab. resp., recovery
	50 "	" " " "
	100 "	" " conv., death 27 minutes.
Goat liver	25 "	Per. irr.
	50 "	" " lab. resp., conv., death 30 minutes.
	100 "	Ruf. coat, " " " " 14 "
Rabbit liver	50 "	Severe symptoms, recovery.
	100 "	Per. irr., conv., death 10 minutes.
Rabbit kidney	50 "	Mild symptoms.
	100 "	Ruf. coat, conv., death 12 minutes.

*Per. irr.—peripheral irritation; ruf. coat—ruffled coat; lab. resp.—labored respiration; par. paral.—partial paralysis; conv.—convulsions.

Tabulation B shows that the toxicity of the tissue cellular protein poison is quite uniform—the average fatal dose, by the intraperitoneal route, being about fifty (50) milligrams.

TABULATION C.

SOURCE OF POISON.	INJECTED INTRAPERITONEALLY.	RESULTS IN RABBITS.
Rabbit liver	380 mgs.	Mild symptoms.
	450 "	Twitchings, lab. resp., conv., death 15 minutes.
Rabbit kidney	450 "	Par. paral., conv., death 30 minutes.
Rabbit heart	350 "	Twitchings, par. paral., lab. resp., recovery.

TABULATION D.

SOURCE OF POISON.	INJECTED INTRAVENOUSLY.	RESULTS IN RABBITS.
Rabbit liver	100 mgs.	Twitchings, per. irr., conv., death 7 minutes.
Rabbit kidney	100 "	" " " " " 12 "
Rabbit heart	100 "	" " " " " 20 "

The results of the experiments reported in Tabulations C and D are interesting from the fact that here it is shown, that the tissue protein poison is toxic for the same species from which it has its origin.

Tabulation E gives the smallest fatal doses of the various protein poisons indicated when these are injected by the intraperitoneal and the intravenous methods in both rabbits and guinea-pigs.

TABULATION E.

SOURCE OF POISON.	INJECTED.	M. L. D. FOR GUINEA-PIG.	M. L. D. FOR RABBIT.
Beef heart	I. V.	0.6 mgs.	90 mgs.
	I. P.	50.0 "	350 "
Goat muscle	I. V.	0.5 "	100 "
	I. P.	50.0 "	380 "
Dog muscle	I. V.	0.75 "	100 "
	I. P.	75.0 "	380 "
Colon poison	I. V.	0.8 "	100 "
	I. P.	75.0 "	500 "
Casein	I. V.	0.6 "	100 "
	I. P.	60.0 "	450 "
Edestin	I. V.	0.6 "	100 "
	I. P.	50.0 "	400 "

The difference in the intraperitoneal and the intravenous dose required to kill a guinea-pig shows (see Tabulation E) that this animal is a hundred times more susceptible to the poison when given intravenously than intraperitoneally; while the rabbit is only four times more susceptible intravenously than intraperitoneally. It would follow then, that one must inject the guinea-pig intraperitoneally with one hundred times, and the rabbit with four times the amount which will actually be absorbed into the circulation.

The guinea-pig and the rabbit are equally susceptible in terms of body

weight when injected with the poison intraperitoneally. It requires about 1 mg. of poison to 4.5 grams of body weight for each species. In contrast it will be noted that by the intravenous route the guinea-pig is killed in the proportion of 1 mg. of poison to 470 grams of body weight; while the rabbit is susceptible to 1 mg. to 18 grams of body weight. It follows then that the guinea-pig is in proportion to body weight about twenty-five times more susceptible intravenously than is the rabbit.

Since the two species, when injected intraperitoneally, are equally susceptible in proportion to their body weights, it follows from the results of the intravenous injections that the guinea-pig takes up poison from the peritoneal cavity less readily than does the rabbit. It must be borne in mind, however, that the guinea-pig is killed by an intravenous injection of one one-hundredth ($1/100$) of the intraperitoneal dose; whereas the intravenous dose required for the rabbit is as much as one-fourth ($1/4$) of the intraperitoneal dose. So then the guinea-pig is twenty-five times more susceptible, so far as the toxicity of the poison to the circulation is concerned, than is the rabbit, but the rabbit absorbs the poison from the peritoneal cavity twenty-five times more readily than the guinea-pig; consequently when given by the intraperitoneal route the poison is toxic for the two species in proportion to body weight.

From the above consideration of dosage, by the two routes in the two species, the following formula may be applied for the determination of the dosage of an unknown specimen of protein poison; granting, of course, that either dose for either species has been determined by trial.

Assume that the minimum fatal dose of the poison by the intraperitoneal route has been determined, then from the formula,

$$\frac{\text{I. P.* for guinea-pig}}{100} = \text{I. V.* for guinea-pig, the intravenous dose is determined.}$$

Since the intravenous dose of the poison is in the proportion of 1 mg. to 470 grams of body weight for the guinea-pig, and 1 mg. to 18 grams for the rabbit, the guinea-pig is twenty-five times more susceptible intravenously than is the rabbit; furthermore by the intraperitoneal method of injection both species are equally susceptible in proportion to body weight. Then the formula,

$$\text{I. V. for guinea-pig} \times 25 \frac{\text{Weight of rabbit}}{\text{Weight of guinea-pig}} = \text{I. P. for rabbit, gives the intraperitoneal dose for the rabbit.}$$

The intraperitoneal dose for the rabbit having been determined, one can now calculate from this the intravenous dose for the same species by the formula,

$$\frac{\text{I. P. for rabbit}}{4} = \text{I. V. for rabbit.}$$
 So then, if one determines by trial

the minimum fatal dose for either route of injection in either species it is evident that the other three doses can be derived from the above formulæ. In conclusion, then, we find from the formula,

*I. P. means intraperitoneal dose; I. V. means intravenous dose.

$$\text{I. P. for guinea-pig} \frac{\text{Weight of rabbit}}{\text{Weight of guinea-pig}} = \text{I. P. for rabbit, that the}$$

guinea-pig and the rabbit are equally susceptible in proportion to their relative body weights. If this is a fact one might reasonably ask: Why is not the rabbit—contrary to experimental findings—as desirable as the guinea-pig for the purpose of demonstrating anaphylactic shock? If minimum fatal doses of specific serum were given intraperitoneally to anaphylactized animals of each species the absorption into the circulation of the guinea-pig of one one-hundredth ($1/100$) of this dose would kill, and in the rabbit the absorption of one-fourth ($1/4$) of the dose would kill. So then there are injected intraperitoneally into the guinea-pig one hundred (100) intravenous doses, while in the rabbit there are injected intraperitoneally only four (4) intravenous doses. Consequently the chances of variation in the absorption of the poison from the peritoneal cavity of the guinea-pig are far less than they are in the rabbit, accordingly when just the minimum fatal dose is given intraperitoneally the chances of producing anaphylactic shock in the two species are one hundred to four (100-4) in favor of the guinea-pig. Aside from the above consideration which deals with minimum fatal doses it may be pointed out, since the body weights of the two species have not been considered in anaphylactic work, that the chances are about sixteen hundred to one (1600-1) in favor of the guinea-pig. In the experiments presented it is shown that the killing dose intravenously for the guinea-pig is two hundred (200) times smaller than that for the rabbit, furthermore since the anaphylactic injections are usually given intraperitoneally, and the relative weights of the two species are not taken into consideration, it is evident that in such tests the guinea-pig has had from sixteen hundred to two thousand chances to develop shock to the rabbit's one chance. It follows that the conclusion from these experiments coincides with Vaughan's statement "that the guinea-pig is the *par excellence* animal for the production of anaphylactic shock."

The following experiments on rabbits, guinea-pigs, and dogs show the effects of the intraperitoneal, intravenous, and intracardiacal injections of protein poison on the clotting of blood, both *in vivo* and *in vitro*.

EXPERIMENT 1.

Normal rabbit—clotting time of blood 8 minutes.

Goat muscle poison injected intravenously 148 mgs. per kilo.

Clotting time after injection.

Bled from heart after	Clotted in
2 minutes	2 minutes
Death 3 "	
7 "	5 "

EXPERIMENT 2.

Normal rabbit—clotting time of blood 5 minutes.

Goat muscle poison injected intravenously 50 mgs. per kilo.

Clotting time after injection.

Bled from heart after	Clotted in
3 minutes	4 minutes
8 "	2 "
20 "	5 "

EXPERIMENT 3.

Normal rabbit—clotting time of blood 9 minutes.

Goat muscle poison injected intravenously 100 mgs. per kilo.

Clotting time after injection.

Bled from heart after		Clotted in
3 minutes		2 minutes
Death 4 "		
7 "		3 "

EXPERIMENT 4.

Normal rabbit—clotting time of blood 9 minutes.

Goat muscle poison injected intraperitoneally 500 mgs. per kilo.

Clotting time after injection.

Bled from heart after		Clotted in
3 minutes		3 minutes
8 "		7 "
Death 10 "		
16 "		4 "
21 "		8 "

EXPERIMENT 5.

Normal guinea-pig—clotting time 10 minutes.

Goat muscle poison injected intraperitoneally 100 mgs. per kilo.

Clotting time after injection.

Bled from heart after		Clotted in
6 minutes		2 minutes
9 "		1 "
Death 10 "		
12 "		4 "
16 "		2 "

EXPERIMENT 6.

Normal dog—clotting time of blood 4 minutes.

Goat muscle poison injected intracardiacally 100 mgs. per kilo.

Clotting time after injection.

Bled from heart after		Clotted in
3 minutes		3 minutes
6 "		2 "
10 "		4 "
Death 13 "		
13 "		4 "
17 "		4 "

EXPERIMENT 7.

Normal rabbit—clotting time of blood 3 minutes.

Casein poison injected intravenously 50 mgs. per kilo.

Clotting time after injection.

Bled from heart after		Clotted in
3 minutes		3.5 minutes
8 "		2 "
13 "		1.5 "

EXPERIMENT 8.

Normal rabbit—clotting time of blood 5 minutes.

Morphine sulphate injected subcutaneously 10 mgs. per kilo.

Casein poison injected intravenously 500 mgs. per kilo.

Clotting time after injection.

Bled from heart after		Clotted in
Death 2 minutes		
3 "		3 minutes
7 "		1 "
10 "		1 "

EXPERIMENT 9.

Normal dog—clotting time of blood 4 minutes.

Morphine sulphate injected subcutaneously 10 mgs. per kilo.

Casein poison injected intravenously 500 mgs. per kilo.

Clotting time after injection.

Bled from heart after	Clotted in
4 minutes	10 minutes
16 "	7 "
20 "	15 "
23 "	90 "
29 "	90 "
52 "	90 "

EXPERIMENT 10.

Normal dog—clotting time of blood 5 minutes.

Morphine sulphate injected subcutaneously 10 mgs. per kilo.

Casein poison injected intravenously 50 mgs. per kilo.

Clotting time after injection.

Bled from heart after	Clotted in
5 minutes	*
9 "	*
19 "	*
30 "	*
50 "	*

*Not clotted in 24 hours.

Tissue cellular protein poisons, in either large or small doses, hasten the clotting of the blood of the rabbit, guinea-pig, and dog *in vivo*. The protein poison from casein differs from these in that it either retards or prevents entirely the clotting of dog's blood.

Edmunds failed to observe any effect upon the clotting of dog's blood when bacillary protein poison was injected. Underhill and Hendrix report that the injection in large doses of protein poison prepared from casein and zein prevent the clotting of dog's blood, this effect being comparable to typical proteose poisoning. Their results are similar to those of experiments 9 and 10 reported in this paper. It will be noted, however, that the smaller dose of casein poison had the greater power of inhibiting the clotting of dog's blood. Although these results are obtained with casein poison it is shown in experiment 6 that the injection of a small dose of goat muscle poison tends to accelerate the clotting of dog's blood. Here we have opposite effects resulting from the injection into dogs of these two protein poisons, thus showing that conclusions drawn from results of experiments with any single protein poison cannot be applied to protein poisons as a group.

In none of our experiments (1 to 8) *in vivo* on rabbits and guinea-pigs with protein poison have we found that the injection retards the clotting of blood; acceleration of clotting, on the contrary, is noted.

Since the tissue cellular protein poison from goat muscle and also the bacillary protein poison, have no effect on the clotting of dog's blood, whereas casein

poison has a decided retarding effect, the difference in the physiological effect may serve to differentiate casein poison from the other two groups.

The following experiments with protein poisons were carried out for the purpose of determining the effect of these poisons on the clotting of fresh blood *in vitro*.

EXPERIMENT 11.

Normal rabbit blood made up to						Clotted in	
.24 per cent goat heart poison						9 minutes	
.6	"	"	"	"	"	10	"
.9	"	"	"	"	"	19	" Jelly clot
1.8	"	"	"	"	"	30	" " "
3.05	"	"	"	"	"	180	" " "
4.15	"	"	"	"	"	*	
4.99	"	"	"	"	"	*	
5.6	"	"	"	"	"	*	
6.4	"	"	"	"	"	*	
7.0	"	"	"	"	"	*	
8.0	"	"	"	"	"	*	
9.0	"	"	"	"	"	*	
Control						3	"

*Not clotted in 24 hours.

In experiment 11 the percentage (0.9 per cent) of goat heart poison necessary to inhibit clotting—if we accept the terminology of Underhill and Hendrix, “that the blood was considered clotted when the containing cylinder could be inverted without the loss of a drop”—*in vitro* was about the same as that of casein poison used on dogs *in vivo* by Underhill and Hendrix, and in which there was no coagulation of blood. It will, however, be noted in the following experiment (Experiment 12) that rabbit’s blood must be made up to 1.8 per cent casein poison in order to prevent clotting. Inasmuch as in both experiments (11 and 12) blood of the same species was employed, it would again appear that the different physiological effects of these two poisons are shown in the clotting of blood.

EXPERIMENT 12.

Normal rabbit blood made up to						Clotted in	
.0005 per cent casein poison						3 minutes	
.005	"	"	"	"	"	3	"
.01	"	"	"	"	"	3	"
.05	"	"	"	"	"	3	"
.12	"	"	"	"	"	5	"
.18	"	"	"	"	"	4	"
.56	"	"	"	"	"	6	"
.61	"	"	"	"	"	5	"
.84	"	"	"	"	"	4	"
1.12	"	"	"	"	"	7	"
1.4	"	"	"	"	"	7	"
1.6	"	"	"	"	"	8	"
1.8	"	"	"	"	"	*	
3.5	"	"	"	"	"	*	
Control						3	"

*Not clotted in 24 hours.

EXPERIMENT 13.

Normal rabbit blood made up to .24 per cent Witte's peptone					Clotted in 2 minutes
1.0	"	"	"	"	2 "
2.0	"	"	"	"	2 "
2.5	"	"	"	"	2 "
3.0	"	"	"	"	3 "
3.5	"	"	"	"	2 "
4.0	"	"	"	"	3 "
4.5	"	"	"	"	3 "
5.0	"	"	"	"	3 "
7.5	"	"	"	"	3 "
10.0	"	"	"	"	4 "
Control					3.5 "

In contrast to the effect of the protein poison on the clotting of rabbit's blood *in vitro* it is shown in experiment 13 that Witte's peptone produces the opposite effect.

EXPERIMENT 14.

Normal dog blood made up to the following percentages of casein poison.	Clotted in 9 minutes	Normal dog blood made up to the following percentages of ox liver poison.	Clotted in 10 minutes
.24.		.24	
.5	8 "	.5	9 "
.75	9 "	.75	9 "
.9	19 "	.9	23 "
1.6	20 "	1.6	25 "
2.0	95 "	2.0	95 "
3.0	*	3.0	*
3.5	*	3.5	*
4.0	5 "	4.0	4 "
Control	7 "	Control	7 "

*Not clotted in 24 hours.

Experiment 14 shows that from 0.24 to 0.75 per cent of ox liver poison and the same percentage of casein poison have but little effect on the clotting of dog's blood; there is a well-defined retardation for from 0.9 to 2.9 per cent; the inhibition is complete for 3.9 to 3.5 per cent; while there is acceleration of clotting with the 4.0 per cent solutions of these poisons. It will be noted that the percentages of these two poisons run parallel to each other as to their influence on the clotting of dog's blood *in vitro*; it will be observed, also, that *in vitro* a much larger percentage of casein is required to prevent the clotting of dog's blood than *in vivo*. If we assume that the ox liver poison has the same effect on the clotting of dog's blood as goat muscle poison *in vivo* (Exp. 6), may we not conclude, since the percentage employed *in vivo* is nearly represented by the .24 per cent *in vitro*, that probably the tissue cellular poisons have similar effects on the clotting of dog's blood both *in vivo* and *in vitro*.

CONCLUSIONS.

1. Vaughan's protein poison can be prepared from tissue cells of the exsanguinated organs of multicellular animals.

2. The tissue cell protein poisons are not only toxic for heterologous species, but also for homologous species.

3. The M. L. D. of the protein poisons—here reported—for the guinea-pig and the rabbit is in proportion to their relative body weights when given by the intraperitoneal method of injection; when given intravenously, however, it is, in proportion to body weight, twenty-five (25) times more toxic for the guinea-pig than the rabbit.

4. Tissue cellular protein poison hastens the clotting of blood from the guinea-pig, rabbit, and dog *in vivo*. The protein poison prepared from casein differs from these in that it either retards or prevents entirely the clotting of dog's blood.

5. Witte's peptone does not prevent the clotting of rabbit's blood *in vitro*.

6. The *in vitro* experiments here reported show that all the protein poisons tested inhibit the clotting of blood from the guinea-pig, rabbit, and dog, in certain percentages.

LABORATORY METHODS

An Automatic Pipette*

BY ALBERT H. ROWE, M.S., M.D., SAN FRANCISCO, CALIF.

THIS pipette has been in use for several months while estimating albumin and globulin in blood sera by Robertson's¹ method. The instrument has proved so satisfactory that it is described in this paper with the hope that it will be found of help in other laboratories where repeated measurements of small amounts of fluids must be made.

For the operation of the pipette a constant supply of compressed air and suction (produced by an ordinary suction pump) is necessary. The photograph shows the arrangement of the apparatus in the size most convenient for use in Robertson's method.

The pipette (a) can be of any style or size. If 1 or 2 c.c. of liquid are to be measured, Folin's modification of Ostwald's pipette is most convenient. If it is desired to measure larger amounts either the ordinary volumetric or Mohr's pipettes may be employed. For Robertson's technic, the pipette is best made of small tubing 2.5 mm. in bore and 30 c.c. in length. The small size of this tube helps accurate measurement and allows the pipette to be inserted into the tubes of 5 mm. bore, thus facilitating their filling. As only equal amounts of different fluids of approximately .5 c.c. volumes are used, it is only necessary to make one mark with a file on the pipette near the .5 c.c. level.

The pipette is held in place by being fitted snugly into a rubber stopper (b) which is inserted into a hole in the cross beam of the frame. To the upper end of the pipette a glass bulb (c) is attached to prevent fluid being suddenly sucked into the pump. To this bulb, a "T" tube (d) is joined, the left arm of which is connected by means of a three way stopcock with the compressed air and the right arm by means of a similar stopcock with the suction pump. These stopcocks (e) are fastened securely to the uprights of the frame. The handles of these stopcocks pass through the center of cardboard dials which bear inscriptions denoting the action of the suction or air when the handles are turned in the indicated directions. Attention is called to the fact that at point (f) on the left dial, a nail is inserted which prevents the handle being turned into a position where there is no escape for the compressed air, which occlusion would blow out the tube connections. If the compressed air contains moisture it is well to run it through a large empty bottle before it comes to the instrument.

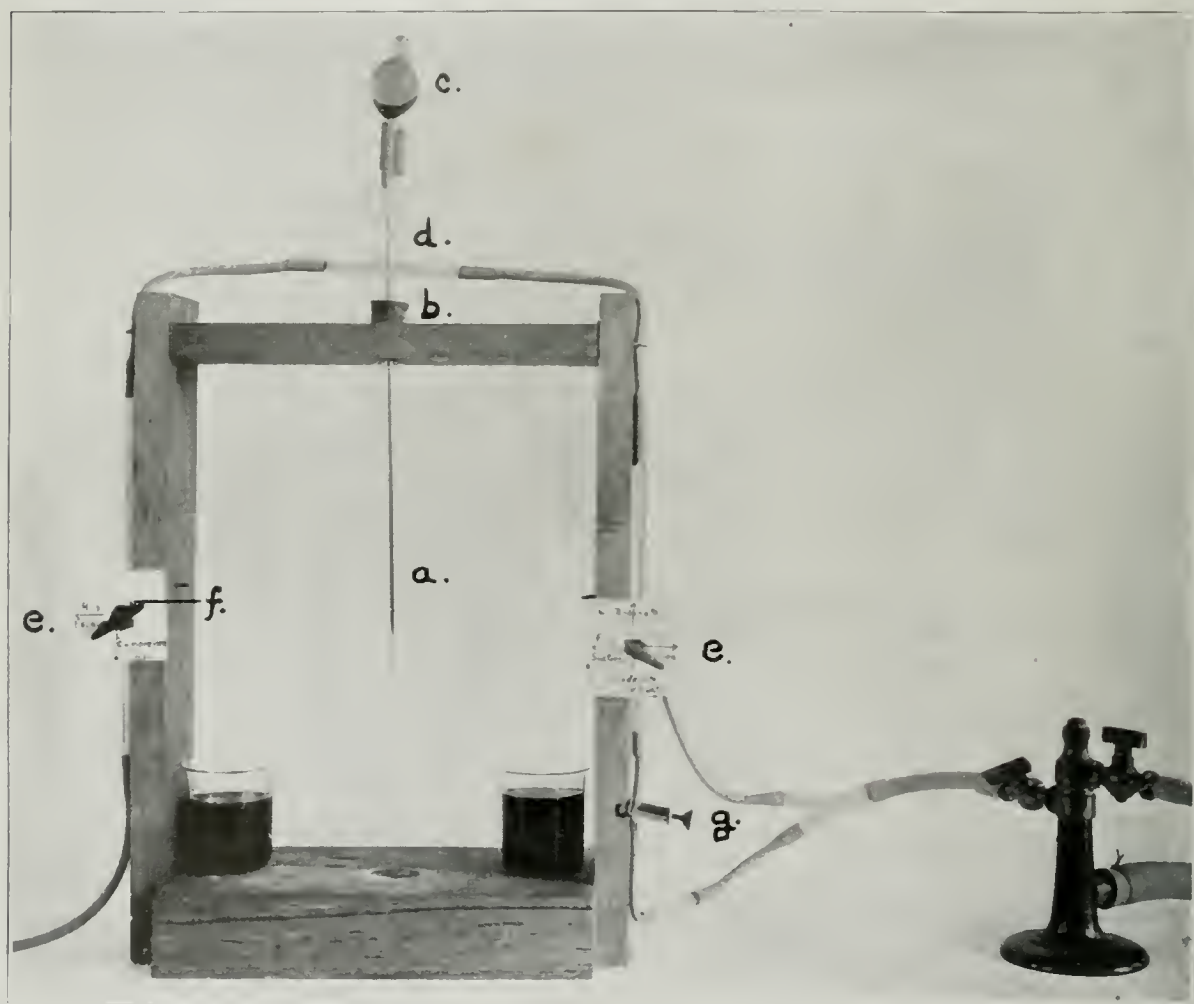
The stopcock on the right has one of its arms filed off so that the remain-

*From the Chemical Laboratory and the Medical Service of the Massachusetts General Hospital, Boston.

¹Robertson, T. B.: Jour. Biol. Chem., xxii, p. 233, 1915.

ing one indicates, by reference to the dial, the action that suction exerts on the pipette. When the handle of the stopcock is turned to the left full suction is exerted on the pipette. In the vertical position, the suction varies according to the degree of closure of the rubber tube by the Hofmann's pinch-cock (g), fastened distal to the dial on the upright of the frame. This pinch-cock can be so regulated that fluid is drawn up into the pipette very slowly. When the handle of this stopcock is turned to the right, suction is cut off and fluid in the pipette is held stationary. It is best to draw the fluid up a little beyond the desired mark and suck it down to the correct level by a piece of filter paper held to the opening of the capillary end.

If compressed air is used to discharge measured fluid from the pipette, it



is done rapidly. But when atmospheric pressure is allowed to enter the upper portion of the pipette by loosening or pulling out the left hand stopcock, the fluid will run out slowly by gravity. The latter method of emptying is often required in Robertson's technic, especially where serum is added to an equal amount of saturated ammonium sulphate solution, by use of which method the serum is introduced so gently that it overlies the sulphate solution and does not mix with it until the tube is shaken.

In Robertson's method, it is frequently necessary, in order not to waste fluid, to clean and dry the pipette before each measurement. This is done by drawing water, alcohol, ether and finally air up and down the pipette. By turning both air and suction into the pipette but having the force of air consider-

ably greater than that of the suction, it is possible to draw the fluid up and down very quickly by merely turning the air on and off.

It is necessary to add that the pipette must be absolutely clean so that no fluid will remain on the inner surface of the tube after it is emptied of its contents. Therefore, it is well to draw cleaning fluid into the pipette, leaving it there when the pipette is not in use. It is even better to have several pipettes so that one can be used which has been in cleaning fluid for several days.

The Schick Test*

BY DON M. GRISWOLD, M.D., M.P.H., DETROIT, MICH.

THE development of the Schick test is one of the recent advances in modern medicine. It is unique in that it does not enter the field of diagnosis or treatment, but is concerned entirely with the immunity or susceptibility to disease.

The diagnosticians and therapists have had their day, and now the immunologists appear to have a real contribution for science.

The so-called skin tests date from von Pirquet's work on tuberculosis. The intradermal test for that disease has traveled as far as propaganda of the fight against the Great White Plague. Skin tests have been developed later for gonorrhoea, syphilis, typhoid and diphtheria. The information sought is the presence or absence of disease in all these tests, except the diphtheria test. In this test the determination of susceptibility or immunity of the patient to diphtheria is the object sought.

Physiological chemistry is a wonderful field, and extensive as our knowledge might seem we have only scratched the surface. To put HCl and NaHCO_3 in a test tube and explain the evolution of gas is comparatively simple. The reason it is simple is because the chemical characteristics of both these substances are very well known. To the person not possessed of this chemical data, the evolution of gas would seem quite wonderful.

And should it not seem equally as wonderful to any of us that we can take certain chemical substances called amboceptor, complement, etc., of which we know nothing regarding the chemical composition, and determine whether our patient has syphilis or not. In dealing with reagents, of which we do not know the chemical character, our test tube results must always be interpreted in the light of our scant knowledge. As one of my former teachers so often said: "Please to remember, gentlemen, a human stomach is not a test tube."

So it seems fitting that such men as von Pirquet, Park, Kolmer and von Schick, should spend many months or years upon tests which leave out of consideration the inanimate test tube, and utilize the human body as a place for physiologico-chemical reactions to be studied. Of course the skin is the part

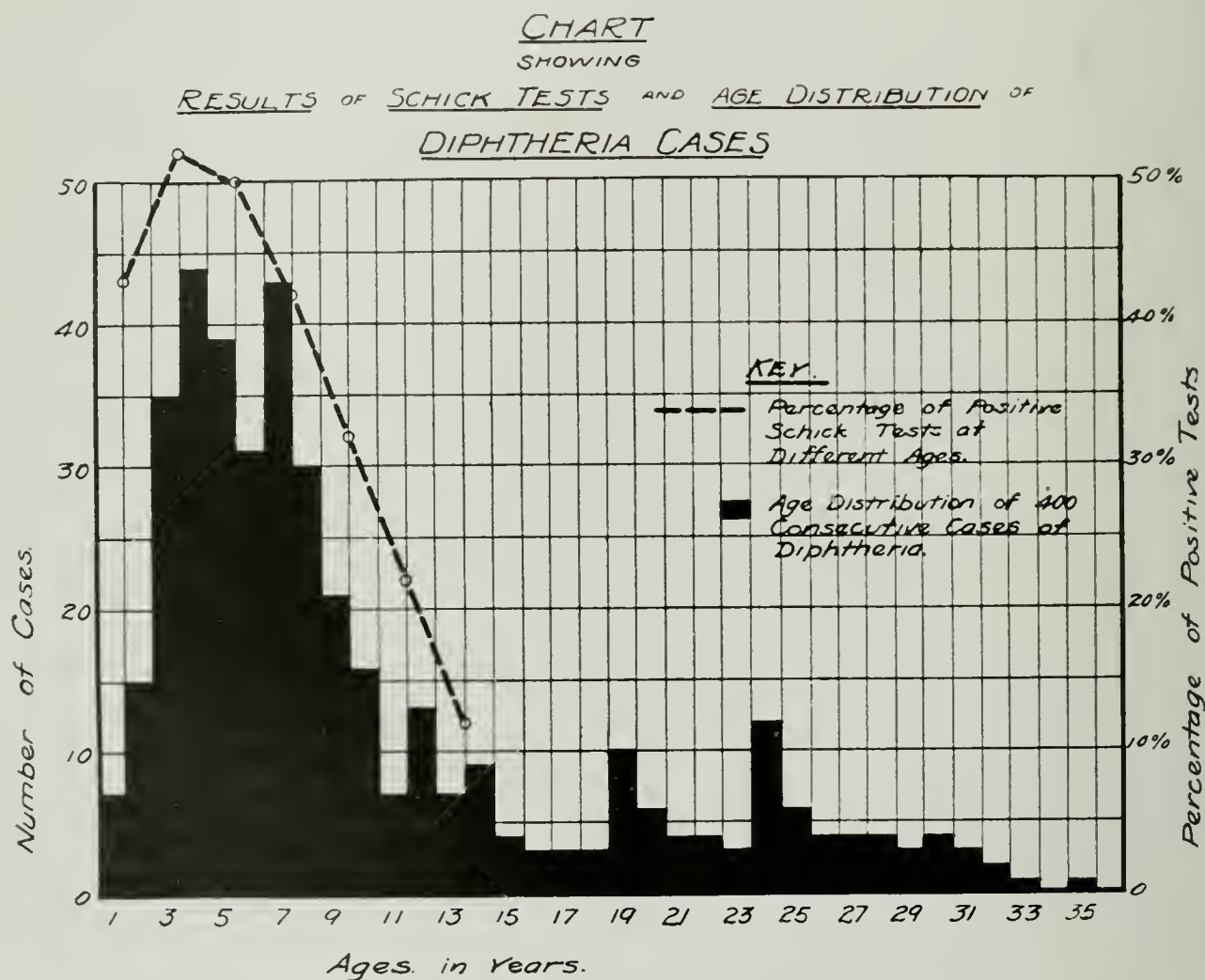
*From the Laboratory of the Detroit Board of Health.

of the body most easily accessible. Inflammation of the skin gives least annoyance to the patient, and is a point most easily determined.

Von Pirquet built the foundation for this line of research when he announced that by applying old tuberculin to abrasions of the skin, characteristic inflammation occurred in tuberculous individuals.

Von Schick, working with the acute contagious diseases in the same clinic, attempted to use the same fundamental principles for work on diphtheria.

Römer has shown that persons who take diphtheria uniformly have less than $1/30$ unit of diphtheria antitoxin per cubic centimeter of their blood serum. He also found that persons who were exposed to diphtheria over long periods of time, such as nurses, internes and doctors, who never took diphtheria, uni-



formly showed more antitoxin in their blood serum than would be expressed by $1/30$ antitoxic unit. From this he concluded that immunity or susceptibility to diphtheria depended upon whether the individual possessed more or less than $1/30$ of an antitoxic unit in each cubic centimeter of his blood serum.

This is a piece of purely scientific data. Von Schick realized how valuable this piece of information would be if the test for $1/30$ of an antitoxic unit per cubic centimeter could be made rapidly and simply enough to allow it to be used in routine work. He found that if $1/50$ of an M. L. D. of diphtheria toxin be diluted to $1/10$ of a cubic centimeter, and the same injected intracutaneously, that persons susceptible to diphtheria would show a red area about the site of the in-

jection, and that immune persons would show no such reaction. The basis of this reaction is, of course, the irritating properties of diphtheria toxin. This irritating toxin injected into the skin of an immune person is immediately neutralized by the antitoxin in the circulating blood, and no reaction is to be seen. When the test is applied to a susceptible person, there being no free circulating antitoxin (or at least less than 1/30 per cubic centimeter), the toxin lays there between the layers of the skin, and in twenty-four to forty-eight hours a slight redness is seen about the site of the injection.

The technic is so simple that it can be acquired by a few trials and can be done very rapidly. In running a large series of these tests, it soon becomes evident that only 30 to 35 per cent of the people are susceptible to diphtheria, and that the remaining 65 to 70 per cent are naturally immune and could not take the disease. This gives the words "naturally immune" a new meaning, because now we know that it is definite diphtheria antitoxin which brings about this immunity and not pleasing generalities of the past "robust constitution," "perfect physical condition," etc.

The percentage of positive Schick tests agrees very closely with the incident of diphtheria for the different ages as is shown by the chart. If we take as proven, that two-thirds of all persons are naturally immune to diphtheria, the question arises at once of the advisability of giving antitoxin promiscuously to all exposures to diphtheria. It stands to reason that two-thirds of the antitoxin will be given to individuals who already have enough to protect them.

The rational procedure would seem to be to give antitoxin to all clinical cases, and a Schick test to all exposures; the following day to read the results of the test and immunize all susceptible persons. The length of time that passive immunity is given by injections of antitoxin has been discussed at length. The only available data being the occurrence of diphtheria in persons immunized by antitoxin.

The Schick test gives us data along this line:

I have found six people who would take a Schick test every second day after they had recovered from diphtheria. The earliest recurrence of a positive Schick was three weeks after 20,000 units of antitoxin. The longest time that 20,000 units gave immunity was six weeks.

To Summarize.—The Schick test seems to give us a quick, easy way of determining whether a person is susceptible to diphtheria. Its use will eliminate needless giving of antitoxin to persons exposed to this disease. It will lessen the danger of anaphylaxis in a community because there will be fewer sensitized persons. It is the greatest single step forward in immunology and serum therapy since the advent of diphtheria antitoxin itself.

Simple Test for Estimating Chlorides in the Urine, Founded on Volhard's Method

BY J. J. SEELMAN, M.D., MILWAUKEE, WIS.

THE following test for estimating chlorides in the urine is accurate to within one-half gram per liter, and can be made in from three to five minutes. Two solutions are made up as follows:

SOLUTION NO. 1.

Anhydrous, crystallized silver nitrate, (C. P.).....	29.055	gms.
25% nitric acid in distilled water.....	900.	c.c.
Cold saturated solution of ammonioferrie alum in distilled water	50.	"
Distilled water, q. s.....	1000.	"

SOLUTION NO. 2.

Ammonium sulphocyanate	7	gms.
Distilled water	1000.	c.c.

The test can be performed with ordinary 1 c.c. pipettes graduated to .01, separate pipettes being used for the urine and for each solution to prevent contamination. I find nipple pipettes more convenient, and have had three pipettes made which I use for this test only. One is a .5 c.c. pipette, one a 1 c.c., and one a 2 c.c. graduated to .05.

Solution No. 2 is purposely made too strong, and must be standardized by adding distilled water in such an amount that exactly the last drop of 2 c.c. of this solution will bring about the end reaction when added to 1 c.c. of Solution No. 1. The end reaction consists of a reddish-brown color, which does not disappear on moderate stirring. If the second last drop produces a discoloration which disappears rather slowly, and the last drop a deep brown color, the solution must be still further diluted, until the discoloration on the addition of the last drop is a light reddish-brown, which does not disappear on stirring fifteen to twenty seconds.

The test proper is performed as follows: Place .5 c.c. of the urine to be tested in a small porcelain dish. Add 1 c.c. of Solution No. 1 and stir about one minute with a glass rod. From a pipette graduated to at least .05 c.c. add Solution No. 2 drop by drop, stirring after each drop until the brown color which develops disappears. When the brown fails to disappear after about fifteen seconds' stirring the end reaction has been reached. Read off the exact amount of Solution No. 2 which has been used to bring about the end reaction. The difference between this and 2 is equal to the number of grams of sodium chloride per 100 c.c. of urine. Example: 1.15 c.c. brought about the end reaction. $2.00 - 1.15 = .85$ gms. per 100 c.c. or 8.5 gms. per liter of urine. If 20 gms. or more per liter are present, as evidenced by the end reaction being obtained with the first drop, the urine is diluted with equal parts of distilled water, which is, of course, allowed for in the subsequent calculation.

It is advisable to maintain three pipettes for this work, using them for no other purpose. They can be marked X, 1 and 2. The first is used for the urine only, the second for Solution No. 1, and the third for Solution No. 2. This prevents contamination of solutions, which would throw them out of adjustment. If desired, pipette No. 2 can be marked to give direct reading of sodium chloride in grams per liter.

A no inconsiderable advantage of this method is the fact that only very small quantities of the reagents are required for each test.

A Rapid and Accurate Clinical Method for the Estimation of Sugar in Small Quantities of Blood

BY J. J. R. MACLEOD, M.B., CLEVELAND, OHIO.

THE attention which has been given to the elaboration of methods for the determination of sugar in small quantities of blood, has been due to the fact that no one method has really been satisfactory. The method may have been accurate enough (e. g., those of Rona and Michaelis, or of Frank), but required too large a quantity of blood to make it practicable in clinical practice, or it may have met the requirements in this regard but have been too complicated and uncertain for use by any others than those who had originally worked it out, or had at least devoted much time and care to learning it (e. g., the so-called micro method of Bang).

It is therefore most important that a simple method which is not only accurate but can be applied to small amounts of blood (1-2 c.c.) should at last be available. The originators of this method are Lewis and Benedict, but it has since been simplified by R. G. Pearce and by Myers and Fine.

Like all other methods for the estimation of sugar, this new one depends on the reducing power of a protein-free filtrate of blood. Instead of using copper salts to indicate the extent of the reduction, however, the conversion of picric into picramic acid is chosen. Since the latter forms a deep red solution, the extent of reduction can be ascertained by using a colorimeter.

The Lewis-Benedict method as modified by R. G. Pearce is carried out as follows: 2 c.c. blood, immediately after withdrawal, is mixed with 8 c.c. of water in a test tube and allowed to become thoroughly laked; 15 c.c. of a saturated watery solution of picric acid is then added, the test tube well shaken and the resulting precipitate of protein removed by filtration. Of the filtrate, 6 c.c. are placed in another test tube and mixed with 2 c.c. more of the picric acid solution and 1 c.c. 10 per cent sodium carbonate solution. Usually two such aliquot portions of the filtrate can be secured, thus permitting of duplicate analysis. The tubes are then placed in an autoclave at a pressure of 2.5 kg. per sq. cm. (or about 20 pounds to the square inch) for 15-30 minutes. After being allowed to cool, the contents of the tubes, now more or less red in color, are

poured into 10 c.c. measuring flasks and the volume in each case made up to 10 c.c. with distilled water. Comparison of the depth of color is now made in a colorimeter (Duboscq) with a standard consisting of either (1) a picramic acid solution of such a strength that the color corresponds to that produced by 0.48 mg. of dextrose under the above conditions (i. e., 0.048 gm. picramic acid and 0.100 gm. anhydrous sodium carbonate dissolved in 1000 c.c. water), or (2) a blank estimation made exactly as above described with a known amount of dextrose (preferably 0.48 mg. in 10 c.c.). By using standards of the above strength the readings on the scale of the instrument are directly proportional to the sugar concentration; e. g., suppose the standard is placed at 10, this must correspond to blood containing 0.1 per cent dextrose (because $6/25$ 2 c.c. or 0.48 c.c. of blood were used), so that if the unknown read 8,* the percentage must be $10/8 \times .1 = 0.125$, and if it read 12 the percentage is $10/12 \times .1 = .083$, and so on.

In Myers' and Fine's modification, the use of the autoclave is dispensed with and simple heating for 15 minutes in boiling water is substituted. To make this possible, however, a lower dilution of the blood is necessary.

The method is carried out as follows:

Two c.c. of blood are discharged into a small test tube containing 8 c.c. of water. After laking, 0.2 gm. of dry picric acid is added and dissolved by stirring with a glass rod. After the protein is completely precipitated, the tube is allowed to stand for several minutes with occasional stirring. It is then centrifuged and the supernatant fluid filtered into a dry test tube through a small thick dry 5-7 cm. filter paper. Three c.c. of the filtrate is placed in a tall graduated test tube, and after adding 1 c.c. of 20 per cent sodium carbonate solution, the tube is placed in a boiling water bath for 15 minutes. After cooling, the volume is made up with distilled water to 10, 15 or 20 c.c., depending on the depth of the color. This is then compared either in a Duboscq or a Hellige colorimeter with a standard. If the Hellige colorimeter is available the wedge is filled with a solution prepared by dissolving 0.100 gm. of picramic acid and 0.2 gm. of anhydrous sodium carbonate in a liter of water. The calculation in this case is carried out by subtracting the colorimetric reading obtained from 100 and multiplying by 0.002 if the dilution was to 10 c.c., or by .003 if to 3 c.c., and so on. We have not personally tested the accuracy of this modification.

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*The scale reads from below up, zero corresponding to such a position of the plunger that none of the colored fluid is being looked through. The lower the reading on the scale, therefore, the stronger the color of the fluid.

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EDITORIALS

The Pharmacological Action of Chlorine Gas

A LONG series of well-known investigators have in the past devoted themselves to the study of the peculiar and striking pharmacological actions of chlorine gas. Conspicuous among these earlier workers are Böhm,¹ Binz,² Lehmann,³ Jurisch,⁴ Herxheimer,⁵ etc.

In the year 1887 Lehmann carried out a long series of animal experiments with chlorine at the Hygienic Institute in Munich. He was chiefly interested in the action of the gas from an industrial standpoint, since in the various manufacturing processes workmen were exposed to fumes of the gas. This work was continued by Lehmann and his pupils for a long period, and a whole series of papers on the general subject of poisonous gases were published by these workers. The method used in general consisted in allowing the animal to breathe for longer or shorter periods of time, air containing various definite percentages of chlorine, etc. The immediate and later (or postmortem) symptoms were carefully studied and described. Lehmann confirmed the earlier work of Böhm and others who had observed that the poisonous action of chlorine is chiefly a local one on the respiratory organs. It was found that a concentration of 1 part in 100,000 parts of air produced an irritation of the eyes, nasal mucosa, mouth, trachea and lungs. This concentration, however, was not sufficient to produce death. It was believed by Binz that small quantities of chlorine like these regularly produced a cerebral

depression, but as the concentration of the gas was increased then the irritative symptoms served to completely overcome and mask the previously observed cerebral action. Later writers appear to have dropped this earlier view of Binz. Lehmann also determined that a concentration of 1 part of chlorine in 10,000 parts of air was capable of producing fatal results in a comparatively short time. If the animal did not die immediately, a short exposure was sufficient to produce lesions from which death would probably result many hours later. It is interesting to note also that Lehmann considered the possibility of sterilizing the lungs and respiratory passages by administering chlorine in sufficient concentration to kill the bacteria. This, however, he quickly gave up.

In 1895 Herxheimer called attention to a new feature of chlorine poisoning. This consisted in the production of a peculiar form of acne. This was characterized by multiple abnormalities of the sebaceous glands. Many of these showed no inflammatory symptoms, but were merely scattered about over the skin as white projecting comedones, some filled, others showing a dark-colored discharge. Other glands were inflamed and contained purulent material. These formed small ulcers and led to deep scar formation which might give the affected area a sieve-like appearance. A number of the glands were enlarged into thick-walled atheromatous cysts, some of which showed no inflammatory changes while others were inflamed and contained more or less purulent material. Furunculosis was often observed. As general symptoms were noted headache, sleeplessness, anorexia, emaciation, anemia, vertigo, etc., briefly, symptoms which apparently depended on the anemia. In later years a good deal of discussion has been carried on regarding the real cause of these lesions. It appears that workmen engaged in the electrolytic production of chlorine are the chief sufferers, and it is still undecided whether the condition is produced by free chlorine or by some other unknown body.

Recently a number of investigators have again taken up the question of chlorine poisoning. In the laboratory, Schäfer⁶ and Hill⁷ especially have done valuable work, while a great many clinical observers have written on the subject.

The symptoms produced by breathing air containing chlorine (1 to 10,000) consist of irritation of the eyes, nose, larynx, and deeper respiratory passages; bronchitis, pulmonary congestion and edema, pulmonary hemorrhages, coughing and pain in the thorax. The amount of irritation produced in the eyes, nose, throat, etc., does not always correspond with the severity of the symptoms which may follow later from the effects of the gas on the lungs, etc. Clinically, there are repeated allusions to the fact that some men appear to stand the action of the gas better than others who have presumably received an equal exposure.

From its chemical nature it seems evident that the immediate action of chlorine must be local. For it is scarcely possible to imagine that it can exist in the free state in such a fluid as blood, which contains many bodies with which it would immediately combine, and which would—unless it were introduced in immense quantities—at once render it innocuous. For chlorine at once displaces hydrogen from its combinations in the proteins and forms hydrochloric acid with the hydrogen set free. And further, it combines with the hydrogen of water

thus liberating nascent oxygen, which then acts on the tissues. These processes are believed to account for the fact that chlorine is a much more powerful disinfectant in moist air than in dry. In the higher organisms all of these reactions probably occur together.

Schäfer saturated Ringer's solution with chlorine gas and then injected the solution intravenously. If only a small quantity were thus introduced no effect was seen, and if a larger quantity, spread over a certain interval of time so that the fluid became well mixed with the blood, were injected, the only result was to produce a quite temporary diminution of blood pressure (preceded by a slight rise) and a slight increase in the depth of the respirations. The fall, Schäfer believed, was due to obstruction of the pulmonary vessels. The diminution in volume of the kidney, which occurred as the blood pressure fell he believed to be passive. These results were produced by the introduction into the jugular vein of a rabbit of 10 c.c. of Ringer's solution saturated with chlorine, the injection being spread over twenty seconds. With inhalation the result is always serious. Even with air containing only 1 per cent of chlorine—although at this dilution no special effect upon either the blood pressure or respiration may be visible for some minutes—a profound change ultimately occurs, and this may show itself with great suddenness. In this case the respirations, while remaining regular both in rate and depth for a few minutes, may suddenly become very deep and in another minute convulsive, prior to complete arrest; while the blood pressure usually falls rapidly and profoundly as the respiration becomes irregular and stops. With 5 per cent of chlorine in the air breathed a fatal result was rapidly and inevitably produced. In none of these latter cases was it possible to revive the animal by artificial respiration. Schäfer considers these effects due to something happening locally in the lungs, since the chlorine which is inhaled cannot be carried to the tissues in a free state. There is moreover, abundant evidence that the tissues retain their vitality even after inhalation of the strongest mixture; for when the body is opened immediately after death, the muscles contract briskly on excitation either directly or through their nerves, and the heart is also responsive to stimulation, and is, indeed, usually seen—especially the auricles—beating spontaneously if somewhat weakly. The only visible change is in the lungs, which even after the shortest exposure to a fatal dose, are intensely red and congested, either all over or in innumerable patches. They are less shrunken than usual, and have a more solid feel, with but little crepitation; nevertheless, even small pieces float in water. On section, the lung is deep red in color, and the tissue seems to be full of blood. There is usually some froth in the trachea and larger bronchi. It is to be noted that if an animal be subjected to a large but nonfatal dose of the gas, and then allowed to live for some time after, other changes than those here described will occur.

The effects which might be produced locally in the lung by an irritant gas-like chlorine are various. It may directly affect the bronchial musculature or the vascular musculature; it may stimulate the mucus-secreting mechanism of the air tubes; it may influence the coagulability or viscosity of the blood within the pulmonary vessels; or it may produce reflex effects by exciting the endings of afferent nerves within the lungs. Schäfer is of the opinion, however, that

the fatal result is due to obstruction in the pulmonary vessels rendering it impossible for the blood to pass freely to the left auricle and ventricle. This latter conclusion he demonstrated by perfusing the lungs in situ in a freshly killed animal. A cannula was tied into the pulmonary artery and the outflow of the solution was collected from the left auricle by means of a second cannula tied into the auricle. The lungs were regularly inflated by an artificial respiration machine. The action of the chlorine was determined by adding Ringer's solution containing chlorine to the solution which was being perfused through the lungs. It was found that even small quantities (1 c.c. of 1 in 10 chlorine Ringer) produced a profound effect upon the pulmonary circulation, the flow through which became greatly slowed almost to complete cessation. Similar results were produced when the chlorine was introduced into the lungs in the air used for the artificial respiration.

In another series of experiments the action of the gas on the bronchioles was studied. Three different methods were used in these experiments (none of these methods seem reliable to the present writer), the final conclusion being that chlorine causes the bronchial tubes to become more permeable (dilate).

Microscopically, sections of the lung showed the pulmonary capillaries gorged with blood, and with an extraordinary amount of edema in the interstitial tissue. Often the edema fluid passed freely into the alveoli, many of which were full of coagulated lymph, some containing blood. Presumably the edema was secondary to the vascular obstruction, but even if this were so it would set up a vicious circle by increasing the obstruction, and this again would increase the edema. That the secretion of mucus did not play an important part in connection with the fatal ending was evident from the fact that death resulted just as inevitably and rapidly after a *præ* dose of atropine as without it. The liver and abdominal organs possessed a normal appearance postmortem.

Clinically, a good many variations from the above description have been recorded because the patients have generally been subjected to the influence of the gas some hours or days before most of the clinical descriptions were made. During this period secondary changes are set up and complicate the original condition. It is remarked by Hill that the action of the gas on the very extensive surface of the lungs (90 sq. metres) is comparable to the effects produced by extensive burns of the skin, and the same general treatment to support strength and lessen shock is required. Just as septic infection of the skin is the sequel of a burn, so pneumonia and bronchitis follow chlorine poisoning.

The clinical course^s of cases of chlorine poisoning has been described as passing through three more or less definite stages:

1. The asphyxial stage.
2. The quiescent or intermediate stage.
3. The bronchitic stage.

The first stage begins as soon as the gas is breathed in sufficient concentration. A sense of suffocation and choking together with greater or less irritation of the eyes, nasal mucosa and respiratory passages lead to strong gasping convulsive efforts at respiration. If the patient is removed from the fumes after several minutes, and if a fatal dose has not been received, the symptoms

will progress, being marked by cyanosis, the escape of a light yellowish frothy discharge from the mouth and nose, collapse, and a variable but usually intense degree of asphyxia. Consciousness is not destroyed unless by asphyxia or impending death. The temperature becomes subnormal, there is restlessness, the pulse is slow and full (except in collapsed cases), the expression is strained and anxious. The patient may sit propped up with his head thrown back gasping for breath, or he may lie with his head hanging over the side of the bed attempting to aid expectoration. The respirations are jerky and hurried and often accompanied by a choking cough. With each inspiration the chest is expanded to its fullest and emphysematous areas are often thus produced in the lung tissues. Auscultation shows the presence of moist sounds of different qualities all over the chest.

After some thirty-six hours the first stage usually passes off and the patient may fall into a sleep from which he awakes feeling much better. This stage may last for perhaps half a day. But after these few hours of comparative quiet symptoms of bronchitis may begin to manifest themselves. Fortunately it appears that this bronchitis stage is not always so severe as would be expected. But death may occur from the effects of the poison and its sequelæ at almost any stage of the intoxication. If the bronchitic stage becomes severe the frothing secretion gives way to a thick greenish mucopurulent expectoration, consciousness may be replaced by delirium, the fever rises, the pulse becomes small and rapid, and the respirations increase in number but become shallow and feeble.

The treatment of these patients presents problems of peculiar and uncommon difficulty. It is obvious at once that the patient needs two things, first a full oxygenation of his blood and second, the removal of the carbon dioxide from his blood. Under ordinary conditions these two processes go on together simultaneously by means of the respiration. But in these cases the ordinary methods of aiding nature in carrying out these processes are greatly handicapped on account of the peculiar condition of the lungs and respiratory passages. For the patient may be continually struggling about and trying to expectorate so that any method of artificial respiration so far tried is used only at a considerable disadvantage. Large quantities of a frothy yellowish fluid clog the air passages and are expectorated with difficulty. Postmortem this fluid is seen to completely fill the small air passages while the finer ones are entirely lost in a condition of intense congestion and edema which affects the lungs as a whole, the congestion involving the mucosa of the entire respiratory tract. The first object of treatment has been to try to get rid of some of this exudation which is drowning the victim. Emetics have proved useful in a large number of cases. Half a pint of salt and water or 8 grains of copper sulphate, followed by large drafts of lukewarm water, have been recommended. Vomiting may be promptly initiated by brushing the back of the throat with a feather or the patient's finger. The act of vomiting has been reported to cause the expulsion of a large quantity of the frothy fluid. As emetics *vinum ipecacuanhæ* and apomorphine hydrochloride do not appear to have been so satisfactory as salt and water. Atropine, amylnitrite, stramonium, chloroform inhalations, etc., do not appear to have been

of any substantial benefit, as indeed might be expected from theoretical considerations. Ammonium carbonate (10 grains every 3 hours) as a "stimulating expectorant" seems to have been of some real benefit. Vinum ipecacuanhæ given along with this has also been said to be of value. Opium may be of value in cases that suffer a good deal from local irritation, etc. The patient should be kept warm and otherwise made as comfortable as possible, and plenty of fresh air should be available. Muscular movements, vigorous treatment, etc., should be avoided when possible on account of the increased demand on the patient's limited supply of oxygen in the blood.

Lastly, and perhaps in the severer cases most important of all, we come to the administration of oxygen. Hill has studied this question very carefully. Various methods and forms of apparatus have been used, but none so far seem to be entirely satisfactory. The cost of oxygen when used by ordinary clinical methods such as administering it through an open funnel held near the mouth and nose, is very great and the benefit is very small. Hill found that when oxygen was administered to himself by an attendant in a London hospital, the oxygen in his own alveolar air was increased by only 1 or 2 per cent. If, however, a loose kind of face-mask were made out of a towel, and the oxygen tube were led under that, and the oxygen were sent in in a sufficient stream to blow away the exhaled carbon dioxide, then 70 per cent of oxygen could easily be obtained in the alveolar air. But a 20 ft. cylinder of oxygen is soon exhausted by these methods. To give oxygen economically and continuously, some closed method must be used. A device by which this may be done has recently been described" in this journal. Hill further found that compressed air was of distinct benefit to animals in this condition. The whole animal was placed in a compressed air chamber in which a pressure of two atmospheres was produced. This not only increased the amount of oxygen (dissolved) which passed into the animal's blood, but it also reduced by one-half the size of the air-bubbles in the frothy liquid which obstructed the air-tubes, thus relieving the obstruction. Artificial respiration, e. g., by the method of Schäfer sometimes has been of distinct help clinically, especially in carrying the patient through a brief crisis. It must not be forgotten that the patient's blood is also loaded with carbon dioxide, and other acids also, such as lactic, are increased in quantity owing to the lack of oxygen. In many instances it has been found that the administration of oxygen for brief periods at a time at intervals afforded distinct and prolonged relief to the patient.

Other diverse forms of treatment,¹⁰ such as bleeding, cupping, leeching, injection of large doses of camphorated and etherized oil, the hypodermic administration of strychnine, sparteine, oxygen, pituitary extract, brandy, adrenaline, sodium cacodylate, etc., inhalations of steam impregnated with compound tincture of benzoin or with eucalyptol, the application of mustard plasters, administration of "ethone," the application of large linseed meal poultices, etc., have been tried. From a theoretical standpoint it is often difficult to understand how a patient who had been severely poisoned by breathing chlorine could live through some of the courses of treatment described. Strangely enough the clinical reports from most forms of treatment are fairly favorable, except for the desperate

cases, the majority of which apparently usually die in the early stage of the poisoning.

It is only to be expected that numerous complications may arise from chlorine poisoning. Some of these have been briefly referred to in recent literature. Bronchitis and pneumonia are the immediate sequelæ of the poisoning. Nephritis as a later development has been frequently mentioned. Fibrosis of the lungs and angina pectoris are also suggested. Pojarisky¹¹ has reported changes in the blood, especially in Bizzozzero's blood plates, and in the walls of the vessels, the endothelium. He believed that from these changes the blood is increased in coagulability. This "thickening" and increased coagulability of the blood is repeatedly mentioned in cases where bleeding was attempted and carried out only with difficulty. Postmortem Pojarisky frequently found miliary hemorrhages in the brain. After the third day he observed such complications as putrid bronchitis, partial pulmonic gangrene, pleurisy, embolism, hemorrhagic infarcts, etc. These may lead to permanent disability even if there is recovery from the immediate effects of the gas.

As a prophylaxis against chlorine poisoning various forms of respirators have been devised. The literature on this phase of the subject, however, appears to be very meager at present. Bollay long ago recommended the use of a large sponge soaked in anilin solution (poison!) which was to be supported before the nose and mouth in order to absorb the gas. Obviously the eyes should be protected from the effects of all concentrated vapors of chlorine. This can be done by a mask with glass windows. Apparently if protection is required against only dilute vapor, then a mask made of several layers (Jurisch suggests 30) of moist flannel may be sufficient. If, however, concentrated vapor is met with, the mask should be moistened with a solution of some chemical capable of neutralizing the chlorine or of arresting its passage into the mouth and lungs. Sodium thiosulphate has been recommended for this purpose, the reaction taking place as follows: $\text{Na}_2\text{S}_2\text{O}_3 + 4 \text{Cl}_2 + 5 \text{H}_2\text{O} = 2 \text{NaCl} + 2 \text{H}_2\text{SO}_4 + 6 \text{HCl}$. The sodium thiosulphate is used in dilute solution and a little sodium hydrate may be added.

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—D. E. J.

The Uric Acid of the Blood

MUCH has been written in the past on the uric acid of the blood, and in no other field, probably, have the reported findings been of so little value. Not only in the medical profession, but also, secondarily, of course, and excusably, among laymen, there has been much loose talk regarding an "increase of uric acid in the blood."

It is only a little more than three years that accurate knowledge as to the uric acid content of the blood has been available. For example, Salecker,¹ in 1909, reported the finding of 5 parts of uric acid in 1,000 parts of blood in a gouty subject, and, as uric acid was not demonstrable by quantitative methods in health, this finding was looked upon as evidence that there is an increased concentration of uric acid in the blood in gout. Brugsch and Schittenhelm² have gone so far as to say that uric acid is lacking in normal blood, while its presence points to the existence of gout.

Folin and Denis,³ in 1912, published their method for the quantitative determination of uric acid in small amounts of blood, and were able to show that this substance is a normal constituent of blood, being present in quantities of 1.5 to 2.5 mg. per 100 c.c. of blood. At the same time they were able to show that in gout, lead poisoning, leukemia and some cases of nephritis, the uric acid may be considerably increased.

More recently, Folin and Denis⁴ have published a comprehensive paper on the diagnostic value of uric acid determinations in the blood. The large accumulations of urate deposits in the joints and cartilages in gout indicate strongly the probability of an augmented amount of uric acid in the blood. But, as a result of several hundred quantitative determinations of uric acid in the blood in different conditions, the authors feel convinced that uric acid determinations alone are not of great value in the differential diagnosis of gout and other arthritides.

Their studies have shown, too, that the uric acid values of blood bear no fixed relation to the values for nonprotein nitrogen. Four possibilities present themselves, as they point out:

"1. Blood in which both uric acid and nonprotein nitrogen are present in normal amounts.

"2. Blood in which, with normal amounts of uric acid, we have greatly increased amounts of nonprotein nitrogen.

"3. Blood giving abnormally high uric acid values with normal amounts of nonprotein nitrogen.

"4. Blood in which abnormally large amounts of both uric acid and nonprotein nitrogen are present."

They then append tables illustrative of each of the four groups. In group I are normal individuals as well as patients suffering from alcoholic gastritis,

¹Salecker: *Deutsch. Arch. f. klin. Med.*, 1909, xcv, p. 353.

²Cited by Folin and Denis.³

³Folin, O., and Denis, W.: *Jour. Biol. Chem.*, 1912, xi, p. 527.

⁴Folin, O., and Denis, W.: *Arch. Int. Med.*, 1915, xvi, p. 33.

cystinuria, diabetes insipidus, arteriosclerosis and cardio-renal disease (one case each), insanity (four cases), and chronic interstitial nephritis (two cases).

In group II, with normal uric acid and high nonprotein nitrogen (50 to 110 mg. per 100 c.c.), are found cases of acute arthritis (three), chronic arthritis (five), nephritis with prostatectomy (one), bone tuberculosis (one), and mitral stenosis (one).

Of great interest is group III, consisting of twelve cases, with high uric acid (3.3 to 5.4 mg. per 100 c.c.) and normal nonprotein nitrogen. Eight of these patients had typical gout. In two patients gout was probable but there were no tophi. Of the two remaining cases, one was a "normal man, many members of whose family have been gouty" and the other a patient who "has passed several vesical calculi consisting of pure uric acid."

The final group IV, with increase in both uric acid (3.3 to 10 mg. per 100 c.c.) and nonprotein nitrogen (59 to 326 mg. per 100 c.c.), is based on observations on fourteen cases. There were four cases of uremia, three of arthritis deformans, one with "weak heart, edema of the lungs, hypertrophic arthritis," one of pneumonia, one with cardio-renal disease, two with nephritis, one with acute gonorrheal arthritis, and one case of "acute gout, tophi, chronic interstitial nephritis."

To quote further from Folin and Denis, "Gout is characterized by abnormally high uric acid content of the blood without any abnormally high accumulation of other waste products (nonprotein nitrogen). Exceptions may, of course, occur; gouty patients may also have nephritis. Nephritis in the gouty is usually of the arteriosclerotic type, a type which, according to our experience, is not accompanied by an excessive accumulation of nonprotein nitrogen in the blood (except in the terminal stages). This rule is, however, unfortunately not without exceptions."

Thus, it is evident that quantitative determination of the uric acid of the blood alone is of little service in the differential diagnosis of gout and other arthritides. To make it of value, a determination of the nonprotein nitrogen of the blood must be made at the same time. If the uric acid alone is increased, the finding speaks strongly for gout. If both uric acid and nonprotein nitrogen are above normal, the blood findings are more suggestive of arthritis of other origin, though the possibility of gout plus nephritis must be borne in mind.

The estimations which Folin and Denis report have been made with their method. The patients have been on a purin-free diet for at least two days before the blood for analysis was drawn.

More recently, Denis⁵ has reported experiments on the effect of diet on the uric acid content of the blood.

Observations have been made on patients after a period of purin-free diet and, again, after a purin-rich diet. The blood was always drawn before breakfast, to avoid the possible effects of a recent meal. It has been found that in normal men "no increase in the circulating uric acid is produced by the ingestion of large quantities of purines. In persons suffering with renal insuffi-

⁵Denis, W.: *Jour. Biol. Chem.*, 1915, xxiii, 147.

ciency, a more or less marked increase in the uric acid content of the blood is produced by high purine feeding." Indeed, this accumulation of uric acid may be found there before there is any rise in the nonprotein nitrogen. Denis concludes that "when the determination of uric acid in the blood is undertaken as a diagnostic test, the insistence on a preliminary period during which no purine-containing foods are consumed is unnecessary, except in cases in which kidney insufficiency exists, or perhaps in the case of persons who habitually consume extremely large quantities of purine-containing foods."

—R. S. M.

The Pituitary in Hibernation

THE dominant manifestations of hibernation are lethargy, storage of fat, decreased tissue combustion, associated with lowering of the body temperature, bradycardia, slowing of the respiration, lowered blood pressure, and insensitivity to painful or emotional stimuli.

Cushing, Crowe and Homans¹ noticed in their cases of experimental hypophysectomy that after the operation the dogs became lethargic and finally died in coma. They noticed that associated with these symptoms there was a noticeable lowering of the body temperature together with slowed respiration, bradycardia, low blood pressure and insensitiveness to external stimuli. They likewise noticed that when not enough gland was removed to produce the fatal cachexia hypophyseopriva that the animals tended to accumulate fat and to lose their sexual activity, and to be drowsy and apathetic with slow pulses and subnormal temperatures, a total symptom picture which simulates that known in human beings as dystrophia adiposogenitalis. These symptoms were relieved by the use of pituitary extracts. So, from the experimental as well as from the clinical sides there was evidence of a distinct similarity between pituitary insufficiency and hibernation.

Recently Cushing and Goetsch² have studied the ductless glands of hibernating woodchucks with the result that they have found very noticeable changes especially in the pituitary glands. They found that the glands were decreased in size and that the cells of the pars anterior had lost their characteristic staining reaction to acid and basic dyes. Also they found that at the end of hibernation the glands enlarge and the cells regain their normal microchemical reactions, and also increase in number.

In this work is given very interesting proof of the fact that what is a normal physiological process in an animal is abnormal in the human even though the appearances are identical. The difference may be that in the one the process is temporary; in the other permanent. It makes one stop and wonder whether it is quite safe to base so many experimental conclusions on the similarities in the morphological or even the chemical reactions in human and animal tissues without knowing the specific physiological relationships.

¹Cushing, Crowe, and Homans: Bull. Johns Hopkins Hosp., 1910 (21) 127.

²Cushing and Goetsch: Jour. Exper. Med., 1915 (22) 25.

—P. G. W.

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ORIGINAL ARTICLES

HAY FEVER AND CERTAIN OTHER LOCAL ANAPHYLACTIC PHENOMENA REFERABLE TO THE RESPIRATORY MUCOUS MEMBRANES*

BY A. PARKER HITCHENS, M.D., AND CLAUDE P. BROWN, M.D., GLENOLDEN, PA.

IN reviewing the history of hay fever, we find that the literature naturally falls into five fairly distinct periods:

First Period.—In the earlier bibliography, long before the first accurate description of the disease by Bostock¹ there are numerous references to periodical attacks of rhinitis and asthma as well as to various idiosyncrasies associated with flowering plants. Among the earliest of these is Botallus² (1565) "for there are many who are attacked with sneezing, by the slightest thing whatsoever, others by merely smelling a rose."† Others mentioned by Sticker³ are Binninger⁴ (1673), Ledelius⁵ (1684), Hünérwolf⁶ (1687), and Constant de Rebecque⁷ (1691). Bostock stated that the earliest reference to which his attention had been called was that of Herberden⁸ (1802). The quotation referred to is probably, "Five patients were attacked violently by this disease for a month every summer; one was afflicted annually for the entire summer; another was never free from it except in the summer."‡ These references are sufficient evidence that hay fever existed for centuries before its recognition as a specific disease, and all attempts to estimate its antiquity are entirely futile.

Second Period.—In 1819, Bostock¹ described a "Case of a Periodical Affection of the Eyes and Chest," so accurately that later writers, even in their

*Presented at the Second Pan-American Scientific Congress, Washington, D. C., Sec. viii, Jan. 5, 1916.

†"Nam plerique sunt qui quaecunque re levissima sternutationis multis agitabuntur, alii ex solo rosae odoratu."

‡"Quinque aegris contigit graviter laborare hoc morbo per mensem omni aestate; alium totam aestatem afflixit quotannis; alius nunquam nisi aestate ab eo liber fuit."

In a translation of Herberden's work published the next year⁹ is the following: "I have known it return in four or five persons annually in the months of April, May, June, or July, and last a month with great violence. In one a catarrh constantly visited him every summer; and in another this was the only part of the year in which it ceased to be troublesome."

most elaborate analyses of symptoms, have been unable to add anything of clinical importance. The attention of physicians thus being called to this affection, reports in a short time began to appear in the medical publications of England, France and Germany. Nine years later Bostock¹⁰ himself added distinct accounts of 18 cases with 10 others "less correctly ascertained." Elliottson¹³ contributed a number of cases and noted many interesting data with regard to the association of symptoms with the apparent exciting causes. From the contributions of Gordon¹¹ and Macculloch¹² we learn that the term "hay fever" was in common use some time before its recognition as a disease by Bostock.

Our information concerning the incidence of hay fever, its relation to age, sex, and condition of life, and its geographical distribution may be attributed in some degree to Elliottson,¹³ but still more to Phoebus.¹⁴

With regard to the etiology, various theories were held and practically every external agency was claimed at first as an exciting cause. Bostock believed his own symptoms to be due to the heat of summer, while other writers held the odors of flowering plants chiefly responsible. The proposal of a bacterial origin by Helmholtz¹⁵ never gained much headway. Pirrie¹⁶ suggested that the disease was of nervous origin, at the same time admitting emanations from plants and various external agencies as exciting causes.

In 1859, Professor Phoebus¹⁴ of the University of Giessen sent out about 400 circulars to physicians, medical societies, etc., requesting information concerning hay fever. His analysis of the replies received remains one of the best statistical studies of the disease, but adds no fundamental fact with regard to etiology. One of the most interesting communications quoted by Phoebus¹⁴ was that of Kirkman who disagreed with the opinion that emanations from *Anthoxanthum odoratum* were a cause (probably *the* cause according to Gordon¹¹), because "I am always attacked at least three weeks before the *Anthoxanthum* is in blossom." Later in his hothouse he noticed, a day or two before Christmas, a single *Anthoxanthum odoratum* in blossom and well loaded with pollen. He rubbed the flower in his hand and sniffed it, whereupon all the symptoms of hay fever appeared immediately, continued for an hour and then left him.

In 1872 and 1876, respectively, Morrell Wyman¹⁷ and Beard¹⁸ published statistical investigations similar to those made by Phoebus. Although Blackley's work had appeared three years earlier, Beard was confident that his theory of a nervous diathesis was conclusive; he said that pollen was merely one of the "debilitating influences, exciting causes purely—and of themselves are powerless to induce, or at least to sustain an attack."

Third Period.—The chaotic state of these earlier opinions could be cleared up only by actual experiment. Without attracting much serious attention at the time, this necessary elucidation was furnished by Charles H. Blackley,^{19, 20} a physician of Manchester, England. In 1873, he published the results of one of the most complete researches in the history of experimental medicine. Blackley, himself a sufferer from hay fever, was at first inclined to agree with Bostock that summer heat was the cause of the disease, but he was led to question the correctness of this view by circumstances connected with a journey to the sea-coast. In the locality of his home at Manchester the hay had already been

gathered in and his attacks of hay fever had ceased; on approaching the seacoast, however, the symptoms reappeared, and later investigation revealed a field of uncut hay much of which was in flower. His attention was directed to the same subject by an incident two years later. Upon examining a bunch of grass brought indoors by one of his children, the cloud of pollen scattering near his face brought on violent sneezing in two or three minutes. Blackley dates his experimental work from this time (1859).

After carefully reviewing all the current opinions concerning the etiology of hay fever, Blackley asked himself the following questions:

1. Can pollen produce the symptoms of hay fever?
2. Does this property belong to all the pollens, or is it confined to the pollen of some one or more orders of plants? And if so, to what natural orders does it belong?
3. To the pollen of which natural order, or of which species of this order, are the actual attacks of hay fever, as they occur in early summer, due?
4. Is this condition or property found in the dried as well as in the fresh pollen?
5. To what special substance in pollen is this supposed action due?

In order to answer these questions Blackley experimented upon the pollen in five different ways: "(1) by applying it to the mucous membrane of the nares; (2) by inhaling it, and thus bringing it into contact with the mucous membrane of the larynx, trachea, and bronchial tubes; (3) by applying a decoction of the pollen to the conjunctiva; (4) by applying the fresh pollen to the tongue, lips, and fauces; (5) by inoculating the upper and lower limbs with the fresh moistened pollen."

Blackley¹⁹ tested on himself the pollens of the grasses and of plants belonging to 35 other natural orders, all of which produced symptoms. He studied microscopically the various pollen grains; furthermore, he counted the grams deposited within a given time upon slides coated with glycerine and carbolic acid. These studies were made at different seasons, indoors and out, in country and in city, at about the level of a man's head and at higher altitudes by means of kites. Blackley demonstrated that symptoms did not appear until the pollen grains had reached a certain number, and that the severity of the symptoms varied in direct ratio with the amount of pollen in the air. He also showed that pollen may travel enormous distances, thus accounting for the fact that the seashore, or even the ocean, does not always afford relief to susceptible persons.

In all this work Blackley himself was the subject of the experiment. He tried in vain to find other hay fever sufferers who would submit to the tests.

In spite of the evidence presented by Blackley, the attention of his contemporaries was so concentrated upon the work of Pasteur and Koch that no suggestion regarding etiology was welcomed unless founded upon bacterial infection. The findings of Helmholz that a vibrio was the cause of hay fever were confirmed by Binz²³ and Patton.²⁴ Heymann and Matzschita,²² however, attempted to straddle the question by suggesting pollen as the carrier of bacteria, especially of streptococci.

Furthermore, the fact that Blackley's work contained no facts of direct therapeutic value naturally detracted from its significance for clinicians. Thus it was not until the successful application of Noon's method of vaccination that the etiologic role of pollen attracted general interest and found final acceptance to the exclusion of all other theories.

Fourth Period.—In 1903, Dunbar²¹ published his work built upon the foundation laid by Blackley. With technic similar to that used by his predecessor, Dunbar experimented upon himself and other susceptible persons with the result that pollen was established as the cause of hay fever—thus the findings of Blackley were confirmed. Dunbar believed at first that only certain pollens were in question. It is probable, however, that the pollen of any plant may be a cause of hay fever; although those varieties found in largest quantities in the atmosphere undoubtedly claim the greatest number of sufferers.

Dunbar was more fortunate than Blackley in having at his disposal the results of all the later achievements in both organic chemistry and immunology—the latter was an unknown science in Blackley's day. Dunbar's work on the chemistry of pollen thus constitutes a real advance in our knowledge of hay fever. In the field of immunology he was able by serological tests to strengthen the evidence in favor of the etiologic role of the pollen proteins. He was the first to establish the possibility of active immunity and to elaborate a specific therapy, but he fell into error by applying to hay fever the discoveries of Behring, Kitasato and others concerning bacterial toxins and antitoxins. Dunbar maintained that hay fever was caused by a true toxin existent in the pollen and that the serum of animals (rabbits and horses) injected with pollen and pollen derivatives possessed true antitoxic power. At present we regard hay fever as an anaphylactic phenomena and consider that the serum elaborated and patented by Dunbar* belongs with the antibacterial serums; in other words, that it depends for its activity upon amboceptor and not upon antitoxin.

Fifth Period.—In 1911 Noon²⁵ reported the results of his work in the laboratory of Sir Almroth Wright on the treatment of hay fever with subcutaneous injections of pollen extracts in minute doses. Curtis²⁶ and Dunbar had previously used plant extracts and pollen extracts, but neither had achieved results sufficiently promising or reliable to encourage a continuation of this work. Noon injected extracts of timothy pollen controlling the doses by ophthalmic reactions; this work interrupted by his untimely death was continued by Freeman.²⁷

Preparation of Pollen Extracts.—Dunbar²⁸ and his associates Prausnitz,²⁹ Kammann,³⁰ Weichardt³¹ and Liefmann³² demonstrated that the protein which constitutes about 40 per cent of the organic substances in pollen is the active agent in causing hay fever. Accordingly all methods in the preparation of material for vaccination must include extraction of this essential protein.

One of the methods used by Dunbar for the preparation of his "Pollen Toxin" is to extract the finely pulverized pollen with 5 per cent sodium chloride solution containing 0.5 per cent phenol, incubating the mixture at 37° C. The undissolved portion removed by centrifugation consists of empty pollen membranes and inactive starch rods. The supernatant opalescent solution contains

*D. R. P., No. 152163.

the dissolved proteins: this is intensely active, even in high dilution, when applied to the skin or conjunctiva of susceptible persons. For use, the extract is diluted with physiological saline solution. Dunbar suggests further purification by precipitation with eight (8) volumes of absolute alcohol or by dialysis. Only the albumens, which constitute about 16 per cent of the total proteins, are toxic, the globulin fraction being entirely inactive.

Subsequent investigators have made their extractions with distilled water and with saline solutions of various strengths. The pollen is sometimes ground with sand; Goodale considers grinding unnecessary.

There are other modifications in technic which are scarcely worth mentioning. In many instances the possibility that the extract is not sterile cannot be eliminated. Standardization is generally considered accomplished by noting the relation between the quantity of fluid and the amount of pollen extracted. This procedure is obviously subject to great variation. Cooke's method of standardization seems to be the most accurate so far and it is the one we have adopted.

Recent Literature.—Among the recent reports of Clowes,³⁵ Lovell,³⁶ Lowdermilk,³⁷ Ulrich,³⁸ Koessler,³⁹ Manning,⁴⁰ Cooke,⁴¹ Wood,⁴² and Goodale,⁴³ the observations of Ulrich regarding intervals between doses are of special interest. He noted that some patients were relieved for much longer periods than others and because of this variability in individuals, he suggested that the intervals be governed in each case by the date of return of the symptoms.

The excellent technic of Cooke with regard to standardization has already been mentioned. His theories regarding the mechanism of hay fever and its treatment are in accord with the latest views on anaphylaxis.

"1. *With regard to antibody formation.*—It must be borne in mind that any form of foreign protein parenterally introduced within the living body, gives rise to the formation of a specific immune or antibody which exists either attached to certain cells or free. When union takes place between protein and free antibody, there is no clinical evidence of a reaction, but when a union takes place between protein and fixed antibody, a reaction takes place, and the nature of this reaction is determined by the type of cell to which the antibody was attached.

"2. *Relation between immunity and anaphylaxis.*—They are qualitatively identical but quantitatively different. In other words, when there is a large excess of antibody circulating free, we have an immune state; and when there is little antibody, and that for the most part attached, we have the sensitized state.

"3. *Duration of immunity.*—Immunity in the sense that free excess antibody once present is always present, does not exist. With cessation of protein injection, the body returns to the anaphylactic state, in which it may remain, or to the state of accelerated capacity to form antibody."

The attempts of Goodale to classify the susceptibility of patients according to natural orders and thus to study this part of the problem systematically, opens up many interesting possibilities not only in hay fever treatment but also in botanical classification.

Work of the Writers.—For obtaining the pollen the flowers are gathered just when pollination has started. The flowers are dried and the pollen collected by means of fine sieves. The pollen itself is thoroughly dried immediately and preserved in the dry state until it is to be extracted.

PREPARATION OF EXTRACT.

1. The pollen is mixed with sufficient physiological saline solution (0.85 per cent) to make a fairly thick paste.
2. The paste is transferred to a porcelain ball mill and ground for 24 hours, or, until microscopic examination shows that the pollen grains are broken.
3. Physiological saline solution is added and the resultant mixture is centrifuged to remove insoluble debris.
4. The extracted protein is purified by precipitation with acetone.
5. The precipitate is dried and thus preserved until needed.
6. For use, the precipitate is dissolved in physiological saline solution. The amount of protein-nitrogen in this solution is determined by the Kjeldahl method.
7. The solution is then diluted so that each cubic centimeter will contain certain fractions of a milligram of protein-nitrogen. The lowest dilution, 1 c.c. of which may be used as the initial dose in treatment, contains 0.0025 mg.
8. The final solutions are preserved from contamination by the addition of 0.25 per cent tricresol and sterilized by filtration. Sterility is determined by careful aerobic and anaerobic cultural tests.

PLAN OF TREATMENT.

Beginning with the minimal initial dose (0.0025 mg.) the treatment may be continued with increasing multiples of this amount according to the needs and the sensitiveness of the patient. The injections are first given at about 5-day intervals, but as soon as the period of relief has been found these intervals are shortened or lengthened, that is, if treatment is necessary during the season.

We consider this better technic than to depend upon ophthalmic reactions which may be dangerous or upon skin tests which merely complicate the procedure for the clinician. In other words, ophthalmic reactions and skin tests bear the same relation to pollen vaccine dosage that the opsonic index bears to bacterial vaccine dosage. By the ophthalmic test Noon and Freeman were able to place pollen vaccination upon a scientific basis similar to that achieved by Wright by means of the opsonic index technic. At present by starting with a dose (of either vaccine) demonstrated to be sufficiently small to do no harm, satisfactory results are obtained without the same control of dosage that was necessary at the beginning.

Our spring extracts contain a mixture of the pollens of red top timothy, rye and orchard grass; the autumn type consists only of ragweed.

These vaccines have been used by ourselves and by other physicians kindly cooperating with us in the treatment of 62 patients.

Of these 62 cases

18 had asthma as a complicating symptom.

Of these:

3.....	no report.
1.....	not relieved.
3.....	considerably relieved.
11.....	entirely relieved.

44 remaining cases.

Of these:

3.....no report.
2.....not relieved.
4.....slightly relieved.
18.....considerably relieved.
17.....entirely relieved.

One patient has apparently been cured; he was treated during two years in both spring and autumn. Another patient who was only partially relieved, was found to be susceptible to wheat, but he was compelled to leave the vicinity before a special extract could be prepared for him.

One patient not completely relieved by the "spring" vaccine was found susceptible to daisy pollen. A special "daisy" vaccine was prepared and the administration of a single dose brought complete relief.

Our results on the whole do not differ greatly from those obtained by others since Noon's first report. In none of these cases have we considered the possibility of a superadded or even possibly of a primary bacterial infection. This point, however, should not be overlooked.

Alexander reports two patients suffering from chronic nasal catarrh which condition was aggravated by pollen during the hay fever season. Treatment with Noon's Pollen Vaccine had no effect. But after bacteriological examination and the administration of *Micrococcus catarrhalis* vaccine, the cases were cured.

We have treated one asthmatic patient with bacterial vaccine, who throughout one winter did not have a single attack, although each winter previously she had experienced many.

Other Anaphylactic Phenomena.—The validity of the so-called idiosyncrasies to the emanations from animals cannot be questioned. Inquiry into the cases of sudden death following the injection of horse serum has shown that many of these persons had previously suffered asthmatic attacks whenever coming in contact with horses.

The manifestations of anaphylaxis after eating certain foods are now well recognized. All these facts lead to the deduction that any protein in the atmosphere may find hypersusceptible individuals who react with sneezing and all the symptoms of hay fever or asthma, or possibly with other more obscure symptoms. A case in point is the effect of bad ventilation (or the lack of ventilation) on certain persons while the other occupants of the room remain unaffected. Rosenau and Amoss³³ have attempted to demonstrate the presence of proteins in the exhaled breath, and although this work as yet lacks confirmation we believe it is a line of research worthy of closer attention.

DISCUSSION.

Hay fever at present is a problem of immunology and of chemistry; and the extension of our knowledge is limited to these two fields unless some unsuspected development may lead to a departure from the present trend of research—a departure such as followed the investigation of anaphylaxis by Rosenau and Anderson.

The demonstration made by Noon that injection of a pollen extract, under the rules laid down by Wright, has immunizing and therapeutic value in hay fever, suggests two important questions:

1. *What essential constituent of the pollen should be contained in the extract?*—This is practically Question No. 5 asked by Blackley, and the work of Dunbar and his associates has gone far toward giving us an answer. However, the lack of a standard method for preparing an extract of maximum diagnostic, immunizing and therapeutic value, clearly indicates the intensive investigation needed in this direction; whether or not the same constituent would possess all these properties would be one point demanding further study.

In certain respects, the method of preparation adopted by us yields a product superior to those prepared by the technic described in recent reports.

a. *Keeping qualities.* Koessler³⁹ among others found his extracts to be worthless after 3 weeks. An extract prepared by the technic given above yielded positive therapeutic results after 2 years; chemical tests according to the Kjeldahl, biuret and Sörenson methods showed no change in protein-nitrogen content nor decomposition with formation of peptones or amino acids.

b. *Uniformity.* The Cooke Method of Standardization certainly appears more rational than that used by Dunbar and others who estimate the strength of the extract by the amount of pollen submitted to extraction. Lack of uniformity in the strength of his vaccines may account for the fear of anaphylactic shock expressed by Lowdermilk.³⁷ None of our patients had more than a slight local reaction which caused no inconvenience.

2. *To what pollens is the individual patient susceptible?*—This is Blackley's Question No. 2. While certain patients are susceptible to the pollen of only one or a very few plants (or at least to a single natural order), others, like Blackley, are susceptible to nearly all plants; there are, of course, all grades between. Goodale has attacked this problem from a practical standpoint by classifying the various pollens with a view to minimizing the number requisite in treatment. Freeman reduced his vaccine to an extract of timothy pollen alone. It would greatly simplify matters for the clinician if in the fall the pollen of a single member of the Compositæ could be applied. A sufficient number of cases has been reported however to show that some patients would not be relieved, even assuming that timothy pollen extract and ragweed pollen extract are adequate for the Gramineæ and the Compositæ respectively. Persons susceptible to rose pollen or to the pollen of certain trees are cases in point.

Although these two questions are the most important at the present time, a third problem is ever present with the practicing physician.

3. *In the present state of our knowledge of hay fever, how can this knowledge be best applied to the advantage of our patients?*—The answer to this question may be considered in two sections; (a) Measures to be taken two or three months before the hay fever season, and (b) Measures to be taken immediately before or during the hay fever season.

a. *Measures to be taken two or three months before the hay fever season.* When the patient can be studied beforehand, a survey of his habitual surroundings should be made. After noting all the flowering plants which might reason-

ably come into question, skin tests should be made with pollens of each of these plants in order to determine which of them are responsible. In this connection it must be remembered that pollens may travel great distances (Blackley); accordingly, a field of grain even several miles away must be taken into account.

It seems scarcely necessary to mention the eliminations that could be made according to the seasonal incidence of the attack. The Gramineæ in the spring, the Compositæ in the fall would receive first consideration.

b. *Measures to be taken immediately before or during the attack.* If the attack has already started, treatment should be begun at once with a vaccine representing the pollens most likely to be responsible for the attack. If the treatment does not give entire relief, an exact diagnosis may be made quite independently of the treatment.

While the ordinary seasonal attacks can be controlled fairly easily, those patients who suffer from earliest spring until latest autumn may present greater difficulties. As stated above, we have treated one patient of this type during two springs and autumns with apparent cure as the result.

As regards dosage we believe that the placing of extracts upon the conjunctiva should be unqualifiedly condemned. Skin reactions may not be quite so exact, but they are adequate for all practical purposes, if indeed any such control is needed, except in diagnosis.

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CEREBELLAR LOCALIZATION IN THE LIGHT OF RECENT RESEARCH*

BY DAVIDSON BLACK, B.A., M.B., CLEVELAND, OHIO.

IN 1870 Hitzig and Fritsch localized certain motor centers in the cerebral cortex by means of electrical stimulation and thus furnished the necessary experimental proof for the theory of cortical localization previously formulated by both Broca and P. Gratiolet.

Since this date many attempts have been made to demonstrate an analogous localization of function in the cortex of the cerebellum. However, indubitable proof of the underlying truth of this concept has only recently been forthcoming.

The cerebellar cortex, unlike that of the cerebrum, presents the same microscopic structure throughout its entire extent. On this account, no facts can be adduced in support of a theory of cerebellar localization on purely histological grounds. It is to researches within the last decade in the fields of comparative morphology, experimental physiology and clinical observation that we are chiefly indebted for our present knowledge of cerebellar localization.

DATA OF COMPARATIVE MORPHOLOGY.

One of the great obstacles in the path of cerebellar research has been the persistent use of a system of nomenclature for the parts of the human cerebellum which is entirely lacking in morphological significance. Another source of confusion lies in the attempt to use the highly specialized human cerebellum as a type with which to compare the simpler cerebella of lower mammals. It is chiefly due to the work of Elliot Smith and Bolk that these obstacles have been removed and a rational method of description introduced.

These authors independently investigated the phylogenetic and ontogenetic morphology of the mammalian cerebellum and were able to recognize a definite plan of cerebellar structure common to all mammals with the possible exception of Monotremes.

Both investigators agree that the mammalian cerebellum consists of two major subdivisions, an anterior lobe and a posterior lobe, separated by a fissure of great constancy termed the *fissura prima*. The classic description of a cerebellum which is composed of a median lobe or vermis and two hemispheres has been rejected as being incorrect.

Figs. 1 and 2 are reproductions of the schemata constructed by Elliot Smith and Bolk to illustrate the morphological arrangement of the parts of the mammalian cerebellum.

The further researches of Bolk have culminated in the formulation by him of a theory of cerebellar localization based upon the data of comparative anatomy.

The parts of the cerebellum are subject to great variation in their relative development in different mammals. For example, in one group (Ungulata) the postero-median lobule is very large while the lobuli ansiformes are small; in

*From the Department of Anatomy, Western Reserve University School of Medicine.

ments can be carried out by the musculature of one arm or leg while the homodynamic organs of the opposite side remain inactive.

If there be representation in the cerebellar cortex of centers concerned in the working of special muscle groups, it is reasonable to suppose that an unpaired center will prove sufficient for the coordination of muscle groups which are necessarily synergic in action. In the case of muscular areas capable of and accustomed to dysynergic action, a paired coordination center must be postulated. However, inasmuch as the limbs are frequently required to act in harmonious coordination one with the other (as for example in walking), an additional unpaired center becomes necessary in their case for synergic coordination.

Working upon the above hypothesis, Bolk successfully demonstrated a definite correspondence between the variations in the development of certain cerebellar lobules and the functional importance of certain muscle groups.

For further details, reference should be made to this author's monograph. The following is a brief synopsis of Bolk's deductions:

The cerebellar cortex is composed of a number of coordination centers some of which are paired and some unpaired.

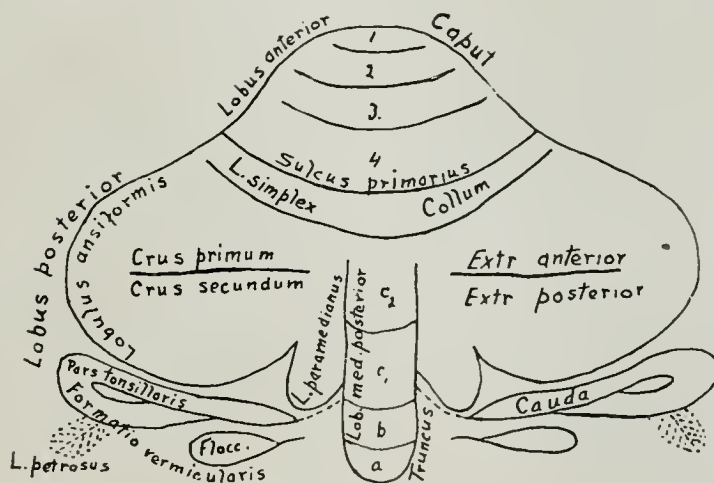


Fig. 3.—Schema of the parts of the mammalian cerebellum spread out in one plane. (After Bolk by Van Rijnberk from Luiciani. Op. cit.) On the right side of the figure the relation of the different lobules to the functional development of the musculature is indicated according to the theory of Bolk noted in the text.

The *lobus anterior cerebelli* contains the coordination centers for the muscle groups of the head (eyes, tongue, muscles of mastication, muscles of expression) and also those of the larynx and pharynx.

The *lobulus simplex* contains the coordination centers for the muscles of the neck.

The upper part of the *lobulus medianus posterior* contains the unpaired centers (centers for synergic movement) for the left and right extremities.

In each of the *lobuli ansiformes et paramediani* are situated the paired centers for the extremities—the arrangement of the centers in each case being homolateral.

In the remainder of the cerebellum the coordination centers for the rest of the trunk and tail region are situated.

These facts have been further summarized and somewhat elaborated in the form of a diagram by Van Rijnberk which is here reproduced as Fig. 3.

For the purpose of correlating the current text descriptions of the human

cerebellum with the rational morphological subdivision of this organ according to Bolk, Figs. 4, 5 and 6 have been constructed. In view of the attached legend further description here is unnecessary.

EXPERIMENTAL DATA.

With the establishment of the homologies of the cerebellar lobes among the various mammalian groups, it became possible to investigate their function experimentally in different forms. Van Rijnberk working in Luciani's laboratory, was the first to test Bolk's theory of localization by means of circumscribed extirpations of certain lobules. The results of his investigations confirmed Bolk's hypothesis and may be summarized in part as follows:

(1) Total or partial extirpation of the *lobulus simplex*: side to side oscillations of the head due to astasia of the muscles of the neck.

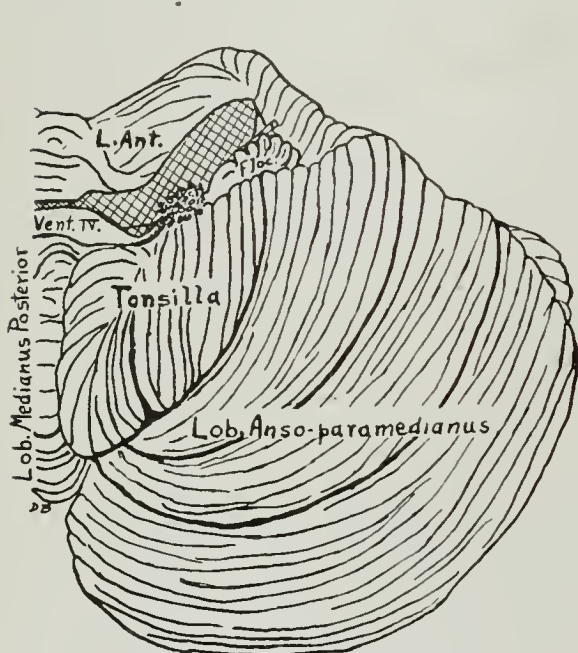


Fig. 4.

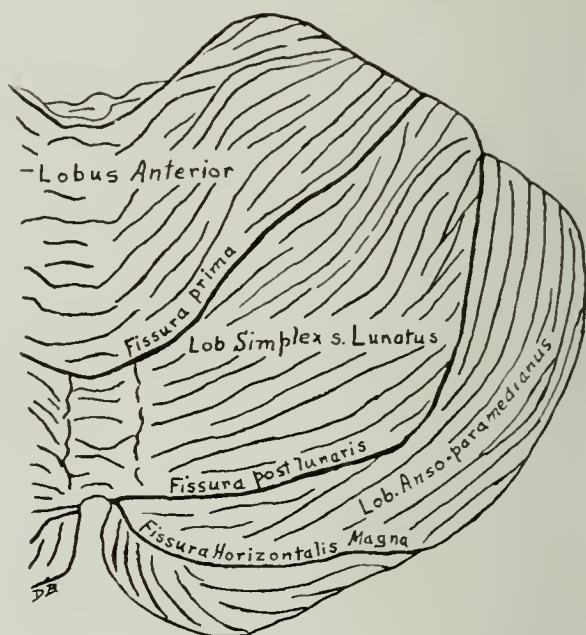


Fig. 5.

Figs. 4 and 5.—Diagrams to represent respectively a ventral view of the left half and a dorsal view of the right half of the human cerebellum illustrating the scheme of subdivision according to Bolk. (From photographs of specimens C. 193 and C. 194 from the Anatomical Museum, Western Reserve Medical School.) The Tonsilla and Flocculus are lobules of the *Formatio vermicularis* of Bolk.

(2) Complete extirpation of the *Crus primum*: homolateral dynamic disturbances in the fore-limb causing in the first irritative stages the assumption of a characteristic position, likened by the author to the military salute. Later occurred considerable dysmetria in the movements of the limb due to muscular atonia.

(3) Extirpation of the *Crus secundum*: homolateral asthenia of the muscles of the hind limb.

(4) Extirpation of both *crura* of *lobulus ansiformis*: marked asthenia and atonia in both fore and hind limbs on the same side as the lesion and the later appearance of a characteristic disturbance in walking termed the "hen's gait."

(5) Extirpation of the *lobulus paramedianus*: rotation on the longitudinal axis of the body associated with pleurothotonus to the operated side.

In each case the symptoms gradually became less marked and eventually almost completely disappeared, owing, according to Luciani, to organic compensation.

These observations of Van Rijnberk have since been confirmed in all their essentials by many investigators working on dogs, monkeys and other animals. In this connection the recent work of Rothmann and of André-Thomas et Durupt on dogs and monkeys should be noted.

These authors point out once more that the destruction of the cerebellar cortex does not give rise to a paralysis; as in man, cerebellar cortical lesions cause a perturbation in the equilibrium of antagonistic muscles: an anisosthenia. They have also carried the investigation of cerebellar localization a step further and have shown that, within the paired centers for the upper and lower extremities described above, there is a definite arrangement of subsidiary centers

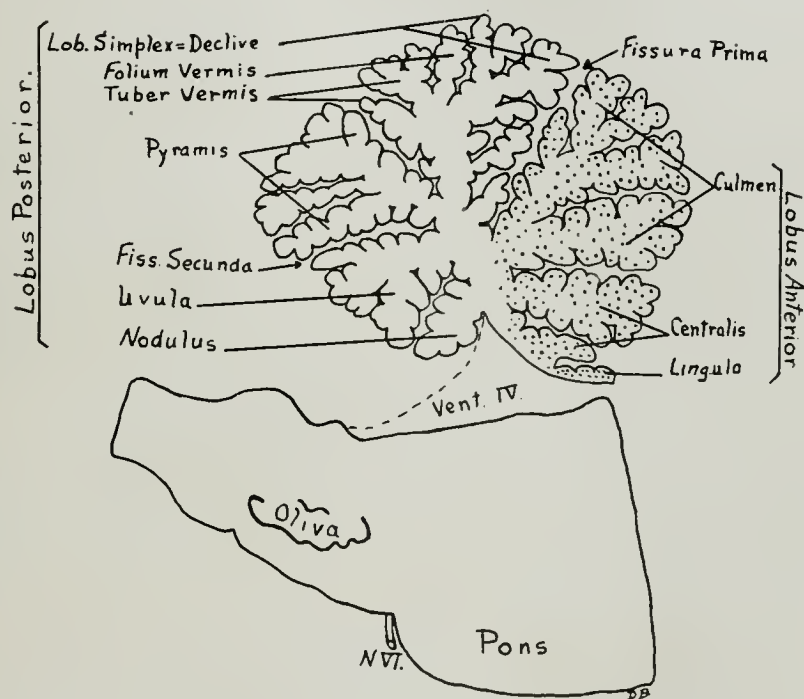


Fig. 6.—Diagram of mesial sagittal section through the human cerebellum to further illustrate the scheme of subdivision according to Bolk. (From specimen C. 192, Anatomical Museum Collection.) The old descriptive terminology for the parts of the so-called "vermis" is indicated for comparison. N. VI, Nervus abducens; Lobus anterior dotted.

for the direction of the activities of antagonistic muscle groups. Each of these secondary extremity centers is related to a single segment or articulation of the corresponding limb and presides over the elaboration of sthenic, tonic and static impulses distributed to definite muscle groups of the segment. As will be seen, these observations are in harmony with those of Barany based upon clinical investigations.

It is important to note that the symptoms of cerebellar deficiency after extirpation experiments, become gradually ameliorated and may, if the lesion be small, finally disappear. This is due to two influences: (1) possible organic compensation by the uninjured parts of the cerebellum itself which has already been mentioned, and (2) actual functional compensation by the purposive and voluntary acts of the cerebrum.

The influence of the cerebrum in the correction of the phenomena of cerebellar deficiency is of great importance. The destruction of one-half of the

cerebellum of a dog gives rise to such marked atonia, asthenia and astasia in the muscles of the same side, that the animal is at first unable either to maintain the erect position or to walk. The severity of these symptoms gradually lessens and after a time the animal learns to stand and to walk once more, though its position and gait are not entirely normal. If a second operation be now performed and the motor cortex of the contra-lateral cerebral hemisphere be destroyed, the symptoms of cerebellar deficiency return in their full severity and the power of standing in the erect position and of walking is permanently lost.

CLINICAL DATA.

The diseases of the cerebellum which have furnished data of a useful nature in this connection are necessarily those caused by circumscribed lesions such as abscess, cyst or regional agenesis. With a positive diagnosis of cerebellar disease the question arises, is it possible to localize the site of the lesion with any degree of accuracy? In this connection reference may be made to the recent work of Rothmann, André-Thomas et Durupt, Babinski et Tournay, and especially to the theory of cortical localization within the human cerebellum, elaborated by Barany.*

On the basis of an extensive series of observations, Barany has described certain clinical tests by means of which he has been able to localize circumscribed cerebellar lesions with a considerable degree of exactitude. It will be of interest here to give a very brief resume of the procedure employed in carrying out these tests, on account of their diagnostic value.

Index Test.—("L'épreuve de l'index"—"Zeigerversuch.")

The patient's eyes being closed, he is asked to execute a simple movement in a given direction with one of his extremities. For example, the forearm being firmly supported, the patient's index finger is extended and brought into contact with that of the observer. The patient is then required to move his finger vertically downward and then to return it to its previous position. The test is repeated a number of times both in the vertical and in the horizontal direction and if any tendency toward deviation from the plane of movement be present, its direction is noted. By slight modifications of the foregoing procedures it is possible to test each of the limb segments in all positions of rotation, pronation or supination.

These tests may be practiced in two different ways: (1) without previously induced nystagmus, and (2) after the production of artificial nystagmus.

In the *normal subject* the following reactions may be observed:

(1) Without previously induced nystagmus, the tests in question are correctly performed—there is no deviation.

(2) After production of artificial nystagmus, a deviation may be noted in the direction corresponding to the slow jerk of the nystagmus. This is termed by Barany the "reaction deviation."

In the presence of a cerebellar lesion certain variations of the above reactions may be observed:

*It is of interest to record in the present connection that this author has but recently been awarded the Nobel Prize in recognition of his distinguished work in this field.

(1) When tested without previously induced nystagmus, a definite deviation of the tested segment may occasionally be noted. This is what Barany terms the "spontaneous deviation."

(2) After production of artificial nystagmus, an absence of the normal "reaction deviations" can be noted. It is this test which has proved to be most useful in diagnosis.

The perturbation or deviation which is observed during these tests, depending upon the direction in which it is produced and the segment of the limb in which it occurs, serves to indicate the exact site of the cerebellar lesion. This statement is based upon the fact that interruption of the cerebellar control of one muscle group leads to an exaggeration of antagonistic muscular action and thus to deviation of the segment during movement in the direction of the hyper-active muscle group.

Barany has thus been able to map out definite areas in the human cerebellar

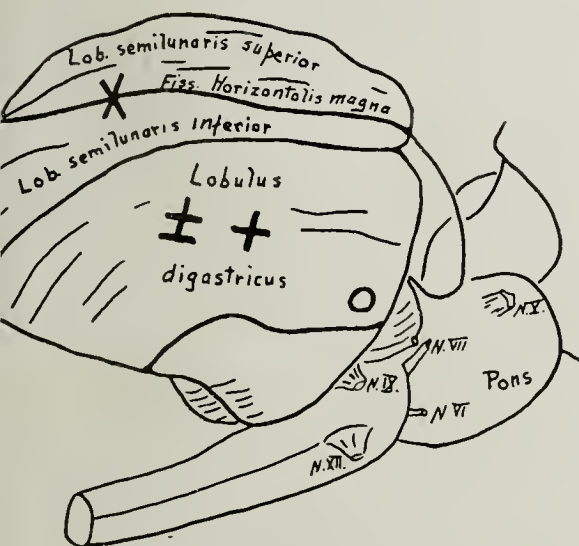


Fig. 7

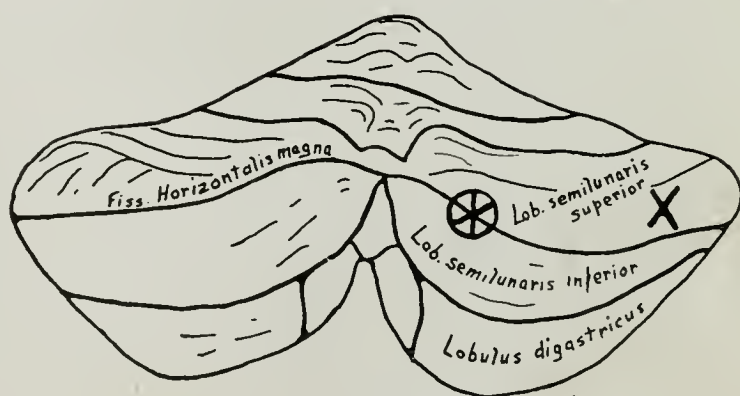


Fig. 8

Figs. 7 and 8 represent respectively the inferolateral and the posterior aspect of the human cerebellum indicating certain cerebellar localizations according to Barany. (After Barany, from André Thomas et Durupt. Op. cit.) See text for description. N. V, Nervus trigeminus; N. VI, Nervus abducens; N. VII, Nervus facialis; N. IX, Nervus Glossopharyngeus; N. XII, Nervus hypoglossus.

cortex, each associated with a special muscular action. The chief deductions this author has made from his observations may be summarized as follows:

(1) There exists an exact localization of function in the cortex of the human cerebellum.

(2) The centers for the extremities are situated in circumscribed areas in the cortex of the hemispheres.

(3) In correspondence with the theory of Bolk and with experimental researches, the centers for the right and left extremities are situated respectively in the right and left semilunar (superior and inferior) and digastric lobules.

(4) Within these chief centers the representation of the limb musculature is spatially determined by the action of its various functional groups and by their position in the limb. Thus, within the arm center, other subsidiary centers are to be recognized concerned in the movements of the limb in the horizontal plane, in the sagittal plane, in rotation, in pronation and in supination; and further, in

each subsidiary center the more minute arrangement of functional areas is in accordance with the movable segments of the limb.*

(5) On the sudden destruction of a center concerned in the movements of a limb in a determined direction (for example, to the right) there is produced a "spontaneous deviation" in the inverse direction (that is, to the left). This "spontaneous deviation" may disappear in time, either through the compensatory action of the cerebrum or of the cerebellum itself.

(6) The chief centers of direction which have been investigated are localized as follows: (vide Figs. 7 and 8).

⊗ A center for the tonus of the musculature concerned in the movements of the right arm downwards, is situated at the medial extremity of the right superior and inferior semilunar lobules. (Abwärtstonus Zentrum.)

× A center for the tonus of the musculature concerned in abduction of the right arm is situated at the lateral angle of the hemisphere in the right superior and inferior semilunar lobules. (Auswärtstonus Zentrum.)

O A center for the tonus of the musculature concerned in adduction of the right hand is situated in the anterior part of the right digastric lobule in the area immediately behind the labyrinth as it lies in the petrous bone.† (Einwärtstonus Zentrum.)

+ A center for the tonus of the musculature adducting the right arm is situated caudad and laterad from the preceding center, in the right digastric lobule.

± Still more laterad and caudad on the right side is situated a center for the tonus of the musculature adducting the right hip.

In the left hemisphere of course a similar arrangement of centers obtains.

It is evident from the foregoing account that the general theory of cerebellar localization as originally formulated by Bolk has been to a large extent confirmed not alone by experimental studies but also by careful clinical observation. Barany's localizations in the human cerebellar cortex remain yet to be confirmed in detail but the importance of his work in thus presenting a possible means of early diagnosis of cerebellar disease cannot be overestimated.

It will be of interest in conclusion to contrast the phenomena of motor localization characteristic of the cerebrum with those of the cerebellum and note the fundamental differences between cerebral and cerebellar control.

The cerebellar cortex has been shown to be practically inexcitable as compared with that of the cerebrum over the motor area.

Muscular representation in the cerebral motor area is chiefly determined by the segmental position of the respective muscles and broadly speaking, the more caudad muscles are represented in the upper portion of the motor area while the most cephalic groups are represented in the lowest areas of the precentral region. On the other hand in the cerebellum, while the grouping of the "tonus centers" has been determined in part by segmental position, their arrangement

*Barany has frequently demonstrated that "spontaneous deviation" (to the right or left in the sagittal plane) may exist in the hand while in the position of pronation but be entirely lacking when the position is changed to that of supination. Such clinical phenomena receive an explanation on the hypothesis of subsidiary centers.

†The subsidiary center for the tonus of the muscles adducting the hand when in the position of pronation, is more medially placed than the center for adduction of the hand when in the position of supination.

within the lobules has been chiefly determined by the functional association of muscular groups.

The cortex of the cerebellum is everywhere concerned in the elaboration of tonic, sthenic and static impulses of a reinforcing nature distributed for the most part homolaterally. A special part only of the cerebral cortex is concerned in the elaboration of impulses of a voluntary, motor, clonic nature distributed heterolaterally.

Destruction of the motor cortex on one side of the cerebrum gives rise to an actual paralysis of a spastic nature in the musculature of the opposite side of the body while destruction of the cortex on one side of the cerebellum causes no paralysis but gives rise to atonia, asthenia and astasia of the musculature on the same side of the body.

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L. Bolk: (1) "Hauptzüge der vergleichenden Anatomie des Cerebellum der Säugetiere, mit besonderer Berücksichtigung des menschlichen Kleinhirns"—Monatschr. f. Psychiat. u. Neurologie, Bd. 12, Heft 5, 1902, pp. 432-467. (2) "Beiträge zur Affen-Anatomie IV—Das Kleinhirn der Neuweltaffen"—Morphol. Jahrb. Bd. 31, 1902, pp. 44-84. (3) "Das Cerebellum der Säugetiere"—Part I, Petrus Camper, Vol. 3, 1905, pp. 1-136; Part II, *ibid.*, pp. 455-598; Part III, *ibid.*, Vol. 4, 1906, pp. 115-194. The theory of cerebellar localization is summarized and the problem of morphological variation in relation to function discussed in the third part of this communication, pp. 153-194. (4) "Das Cerebellum der Säugetiere"—Jena, 1906. This is a collection in book form of the researches published in Petrus Camper in three parts.

G. Van Rijnberk has published critical reviews of the current literature on cerebellar localization up to 1912—(1) "Die neueren Beiträge zur Anatomie und Physiologie des Kleinhirns der Säuger," Part III, Folia Neurobiologica, Bd. I, 1907, pp. 535-551. (2) "Weitere Beiträge zum Localisationsproblem in Kleinhirn." *ibid.* Bd. 6, 1912, pp. 143-170. Reference should also be made to *Luciani's* discussion of cerebellar localization in "Human Physiology," Vol. III. English translation from 4th Italian Ed., Macmillan & Co., 1915—pp. 473-485.

André-Thomas et Durupt, in their book "Localisations Cérébelleuses" (Paris, 1914), present the results of their researches upon dogs and monkeys together with an interesting and detailed review of the subject in all its phases and an extensive bibliography.

Babinski et Tournay in their communication "Le Symptomes des Maladies du Cervelet et leur Signification" read at the meeting of the 17th International Congress of Medicine in London have discussed the clinical aspect of this problem. An extensive clinical bibliography is also given in this report—Transactions, Section XI, Part I, 1912—pp. 51-58. The paper by *M. Rothmann* on "The Symptoms of Cerebellar Disease and Their Significance" (*ibid.*, pp. 59-83) should also be consulted in this connection.

R. Barany has published a number of his important clinical observations under the title, "Lokalisation in der Rinde der Kleinhirnhemisphären des Menschen"—Wiener klinische Wochenschrift, No. 52, Dec. 26, 1912. His technic is described and a brief summary given in the papers by Babinski et Tournay and by André-Thomas et Durupt, cited above.

REGULAR ECTOPIC RHYTHMS*

BY FRANK N. WILSON, M.D., ANN ARBOR, MICH.

IN the normal heart the sinoauricular node, a specialized structure which lies in the floor of the sulcus terminalis of the right auricle, has a higher degree of rhythmicity than any other portion of the cardiac tissue and it is in this node that the normal cardiac rhythm has its origin. Under abnormal conditions, however, some other part of the heart may initiate stimuli more rapidly than the sinus node or may be functionally separated from it by conduction changes and may thus become the cardiac pacemaker. Rhythms which arise outside of the sinus node have been called ectopic rhythms. Lewis¹ has long insisted that such rhythms should be divided into two classes, homogenetic ectopic rhythms and heterogenetic ectopic rhythms. In the first type the impulses are supposed to be generated by physiologic, in the later type by pathologic processes.

REGULAR ECTOPIC RHYTHMS OF THE HETEROGENETIC TYPE.

Heterogenetic rhythms are recognized by the following characteristics: (1) The impulses which give rise to them are formed at a very rapid rate. This may lie anywhere between 140 and 370 per minute. (2) The rate is remarkably constant and is little or not at all influenced by procedures which influence the normal cardiac rhythm such as change of posture, exercise, or stimulation of the vagus nerves. It is true that such rhythms sometimes cease abruptly upon the application of various therapeutic measures which involve vagus stimulation but the cessation of the abnormal rhythm is not usually preceded by any gradual slowing and the results of vagus stimulation are very inconstant. (3) There is a marked and abrupt change in heart rate at the onset and at the end of the abnormal rhythm. (4) The abnormal rhythm occurs as a rule in paroxysms which cease abruptly and which are followed by a post-paroxysmal pause analogous to the pause which follows a premature systole. In fact such rhythms are believed to be made up of a series of premature beats arising at the same point. They probably arise only when the physiologic integrity of some small area of the heart muscle has been interfered with either by an organic lesion, by some toxic agent, or by nutritional changes.

PAROXYSMAL TACHYCARDIA.

Clinically, we divide regular rhythms of the heterogenetic type into two classes, paroxysmal tachycardia and auricular flutter. Paroxysmal tachycardia usually arises in the auricular muscle, less commonly in the ventricular muscle or in the special muscular structures which unite auricles and ventricles. The heart rate during the paroxysm usually lies between 140 and 220 per minute. An example of an attack of paroxysmal tachycardia of auricular origin is shown in Fig. 1. It exhibits the typical features of a heterogenetic rhythm; the rapid rate (150 per minute), the abrupt and marked change in heart rate at its ter-

*From the Department of Internal Medicine, University of Michigan.

mination, and the post-paroxysmal pause. The ectopic nature of the rhythm is shown by the abnormal form of the P-wave of the electrocardiogram. This is inverted and occurs between the R- and P-waves of the ventricular complex. The last ventricular complex is not modified by one of these inverted P-waves, which indicates that the last ventricular contraction occurred in response to the auricular contraction which coincided with the previous ventricular systole. The prolongation of the P-R interval in combination with the rapid heart rate caused auricles and ventricles to contract simultaneously and thus produced the positive venous pulse seen in Fig. 1. This increase in the length of the P-R interval is common in paroxysmal tachycardia and is probably due to fatigue of the junctional tissues as a result of the rapid heart rate. A diagram of the cardiac mechanism is shown in Fig. 1, the heterogenetic character of the ectopic rhythm

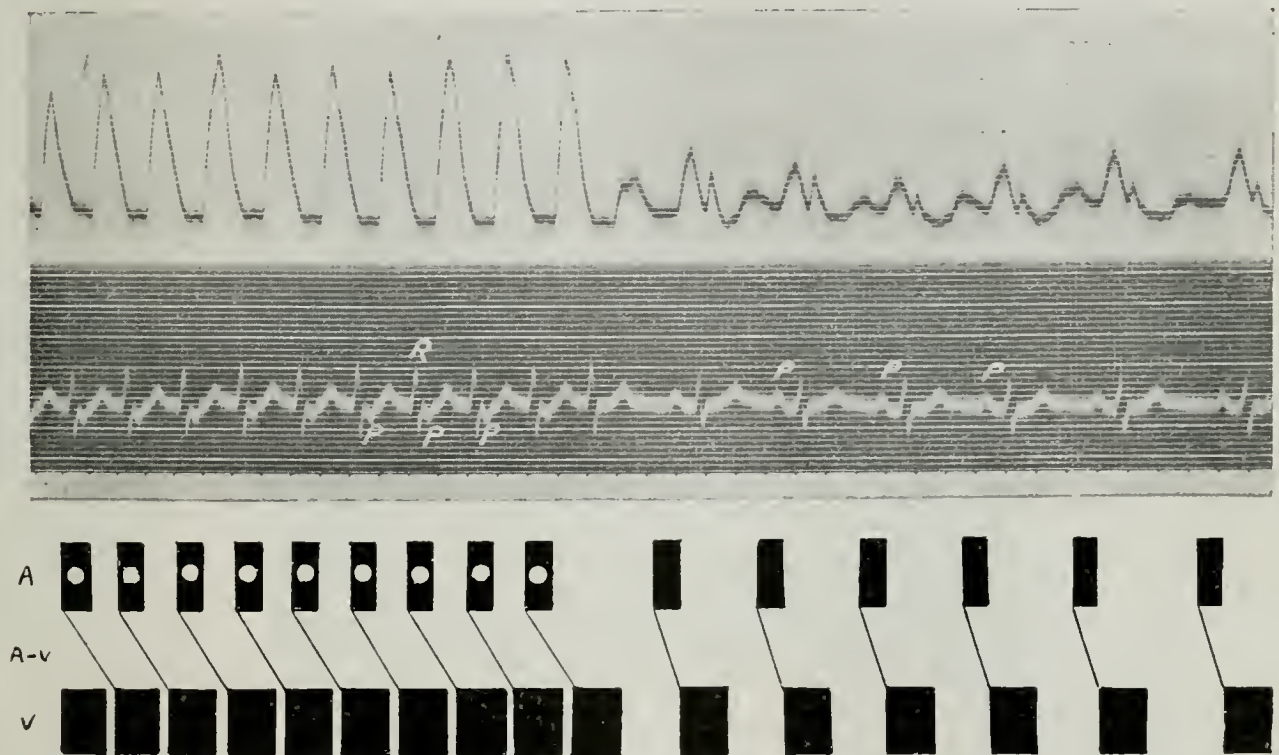


Fig. 1.—Lead II. The end of an attack of paroxysmal tachycardia. Heart rate during attack 150 per minute. Heart rate after attack 86 per minute. The change in heart rate at the end of the abnormal rhythm is abrupt and there is a post-paroxysmal pause. The P-wave is inverted during the abnormal rhythm and occurs between R and P' of the ventricular complex. There is a positive venous pulse during the paroxysm. The cardiac mechanism is diagramed below the tracing. The heterogenetic character of the ectopic rhythm is represented in the diagram by the white centers of the auricular rectangles which correspond to the inverted P-waves.

being represented by the white centers of the rectangles which correspond to auricular systoles.

AURICULAR FLUTTER.

Auricular flutter differs from paroxysmal tachycardia of auricular origin in the following particulars: (1) The auricular rate is more rapid and lies between 200 and 370 per minute. (2) Either because of the very rapid auricular rate alone or because of a depression of the conductivity of the junctional tissues in addition the ventricles do not respond to every auricular contraction. When the patient first comes under observation the ventricular rate is most often one-half the auricular. Vagus stimulation does not influence the auricular rate but slows the ventricles by increasing the grade of block. Digitalis has a

similar effect and it is common after the administration of this drug to find that the ventricular rate has been reduced to one-fourth of the auricular. In large doses digitalis frequently transforms auricular flutter into auricular fibrillation and finally this may be followed, when digitalis is discontinued, by the return of the normal rhythm. (3) Attacks of auricular flutter are usually much longer than those of paroxysmal tachycardia, their duration being measured in months or years rather than in hours or days. The essential features of this disorder are shown in Fig. 2. The auricles of the patient from whom this record was obtained were beating at the rate of 240 per minute while the ventricles which responded to every fourth auricular contraction were beating but 60 times per minute. Occasionally the ventricles responded irregularly to every third, fourth, fifth, or sixth auricular systole. The P-waves of the electrocardiogram have an abnormal outline indicating that this abnormal rhythm is ectopic. Each P-wave is accompanied by an a-wave in the venous pulse. The auricular rate was very constant from day to day and was not influenced by vagus stimulation. This fact together with the very rapid rate of impulse formation indicates sufficiently well the heterogenetic character of this ectopic rhythm.

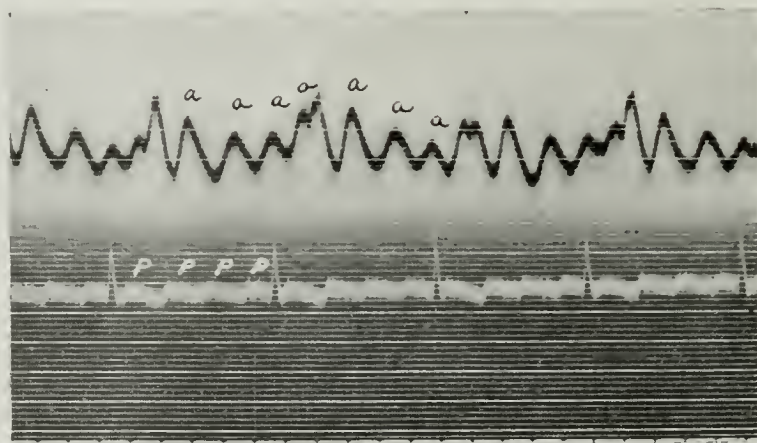


Fig. 2.—Lead II. Auricular flutter. The auricular rate is 240 per minute, the ventricular rate 60 per minute. The P-waves are abnormal in form. Corresponding to each P-wave there is an a-wave in the venous pulse.

HOMOGENETIC ECTOPIC RHYTHMS.

Homogenetic ectopic rhythms are recognized by the following characteristics: (1) They are of comparatively slow rate. (2) The change in heart rate at their onset and at their end is gradual and is usually not very marked. (3) The rate of these rhythms is influenced like the normal rhythm by exercise and by stimulation of the extracardiac nerves. (4) There is no pause following the abnormal rhythm similar to that seen at the end of an attack of paroxysmal tachycardia.

Ectopic rhythms of this type lie dormant everywhere in the heart although practically we have to deal only with those centers which lie in the specialized tissues of the atrioventricular junction whose rhythmicity is greater than that of the undifferentiated cardiac muscle elsewhere. All of these centers remain quiescent until an increase in their rhythmicity or a decrease in the rhythmicity of the sinus-node enables that one whose inherent rate is highest to assert itself. Since most homogenetic ectopic rhythms have their origin in the junctional tis-

sues they are usually referred to as atrioventricular rhythms. Such rhythms may arise spontaneously, most often during the course of certain acute diseases especially diphtheria² and acute rheumatic fever.³ More often they may be produced by stimulation of the vagus.^{4, 5} They may also occur after the administration of digitalis.⁵ We have recently been able to show that such rhythms may be produced in the majority of young adults by vagus stimulation between eight and fifteen minutes after the administration of one-fiftieth of a grain of atropin sulphate.⁷ This drug appears to increase the susceptibility to atrioventricular rhythm by a selective action upon the vagus endings in that it paralyzes those that are found in the auriculoventricular node before it paralyzes those that lie in the sinus node. We have been able to obtain a large number of atrioventricular rhythms by this method. They are usually of short duration and are



Fig. 3.—Lead III. An A-V rhythm of type 1, with the transition to the normal rhythm. During the A-V rhythm the P-wave is inverted and the P-R and a-c intervals are slightly reduced. In the third cycle the P-wave is invisible although there is a definite a-wave in the venous pulse. The absence of the P-wave is due to the fact that two excitation waves met in the auricular walls and neutralized each other electrically. The cardiac mechanism is diagramed below the tracing. This A-V rhythm originated high up in the junctional tissues or possibly in the coronary sinus region.

not accompanied by symptoms except that palpitation is occasionally experienced when auricles and ventricles contract simultaneously. For convenience in their description atrioventricular rhythms may be divided into three types; type 1 in which the P-R interval is present but reduced, type 2 in which the P-R interval is zero, and type 3 in which there is an R-P interval.

Atrioventricular rhythm of type 1 is illustrated in Fig. 3. The abnormal rhythm is comparatively slow, there is no abrupt change in heart rate and no pause at its termination. These facts indicate its homogenetic character. The P-wave of the electrocardiogram is inverted and the P-R and a-c intervals are both slightly reduced indicating that the ectopic rhythm originated in the upper levels of the junctional tissues. The cardiac mechanism is diagramed below the tracing.

Atrioventricular rhythm of type 2 is illustrated in Fig. 4. This ectopic

rhythm displays the same characteristics as that of Fig. 3 except that the P-wave cannot be made out during the abnormal rhythm. There is a tall wave in the venous pulse, however, which indicates that auricles and ventricles contracted simultaneously. The P-wave is buried in the R-wave and the P-R interval may therefore be given as zero. Such rhythms arise in the central portion of the junctional tissues.

Fig. 5 illustrates atrioventricular rhythm of type 3. Here again the abnormal rhythm has the characteristics which place it in the homogenetic group. In this instance, however, the P-wave which is inverted falls behind R and there is an R-P interval of .175 seconds. Such rhythms originate in the lower levels of the junctional tissues.

THE TRANSITIONS BETWEEN NORMAL AND ATRIOVENTRICULAR RHYTHM.

The transitions between normal and atrioventricular rhythms are of interest since they illustrate very well the essential differences between homo-

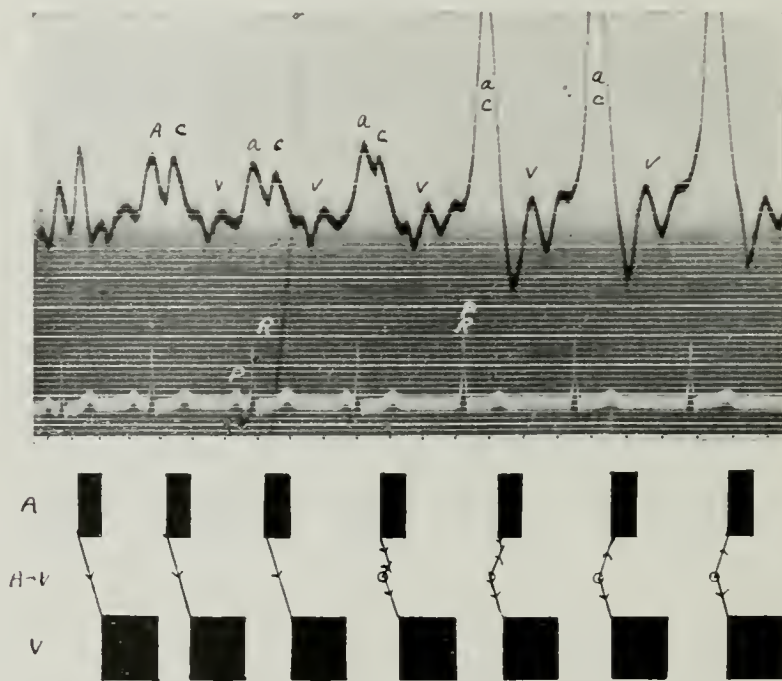


Fig. 4.—Lead II. An A-V rhythm of type 2, with the transition from the normal rhythm. At the beginning of the abnormal rhythm P without changing its form gradually approaches and disappears within the R-wave. During the abnormal rhythm (last three cycles) P is invisible but there is a tall wave in the venous pulse indicating that auricles and ventricles contracted simultaneously. This rhythm originated in the central portion of the junctional tissues, presumably in the region of the A-V node. The cardiac mechanism is diagramed below the tracing. Note that in the fourth and fifth cycles there is an interference of two excitation waves in the junctional tissues and that in these two cycles the auricles responded to the sinus node and the ventricles to the center in the junctional tissues.

genetic and heterogenetic ectopic rhythms. A transition from atrioventricular rhythm of type 1 to the normal rhythm is shown in Fig. 3. There is very little change in heart rate at the end of the abnormal rhythm. The P-wave of the third cycle which marks the transition from abnormal to normal rhythm is isoelectric. In this instance the two excitation waves, one from the sinus node and one from the junctional tissues, neutralized each other electrically. More often the P-waves which are due to the interference of two oppositely directed excitation waves in the auricular muscle are either small and upright or diphasic (Fig. 5).

When transitions from normal rhythm to A-V rhythm of type 2 occur (Fig. 4) the P-wave of the normal rhythm does not change its form but there is a gradual shortening of the P-R interval so that P gradually moves up to and disappears in R. No deformed or inverted P-waves are seen since the excitation wave from the junctional tissues does not reach the auricles until the beginning of the R-wave. The shortening of the P-R interval with no change in the shape of the P-wave which occurs during such transitions indicates that for a short period the auricles respond to the sinus node while the ventricles respond to the atrioventricular node.

This period of auriculoventricular dissociation is much longer as a rule in transitions from the normal rhythm to an atrioventricular rhythm of type 3. In Fig. 5 it will be seen that the P-wave gradually approaches the R-wave and finally appears after R in the sixth cycle without changing its form. Indicating that during this period the auricles are responding to the sinus node as in the normal rhythm. In the seventh cycle, however, the P-wave is deformed because in this instance there was an interference of two excitation waves in the auricular muscle. After this transitional P-wave auricular systole is represented by an inverted P which means that in the last two cycles seen in the figure the auricles responded

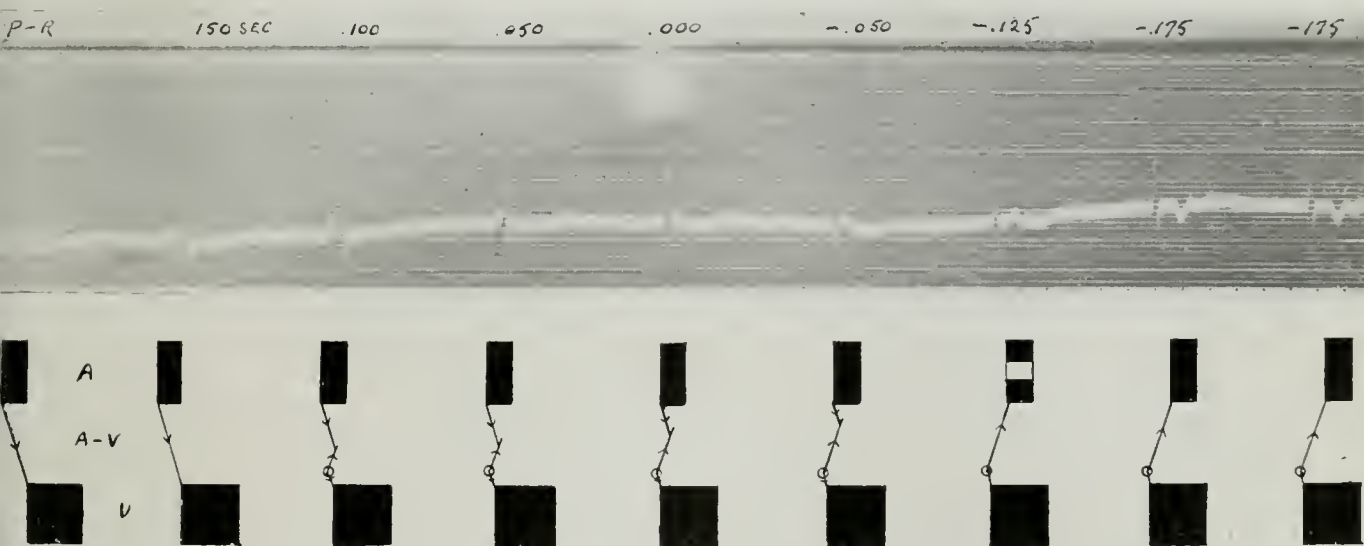


Fig. 5.—Lead II. A transition from normal rhythm to an A-V rhythm of type 3. The P-wave gradually approaches R and finally appears after R in the sixth cycle without changing its form. From cycle three to cycle six the auricles responded to the sinus node while the ventricles responded to a center low down in the junctional tissues, probably in the main stem of the His bundle just above its bifurcation. The two excitation waves met during this time in the junctional tissues. In cycle seven the two excitation waves met in the auricular walls and the P-wave of this cycle is deformed. In the last two cycles the auricles responded to the junctional tissues and the P-wave is inverted. There is an R-P interval of .175 seconds during the A-V rhythm while the normal P-R time is only .150 seconds in this case.

to the center in the junctional tissues. When the transition from normal to A-V rhythm is not complete short periods of dissociation similar to that seen in Fig. 5 may occur alone. In such cases the fact that the P-wave is of the normal form while the P-R interval is reduced should lead to their correct analysis.

THE FORM OF THE P-WAVE AND OF THE VENTRICULAR COMPLEX IN ATRIOVENTRICULAR RHYTHM.

From the analysis of a large number of atrioventricular rhythms obtained by vagus stimulation after the administration of atropin we have found that in

A-V rhythm of type 1 the P-wave is inverted in leads II and III and upright in lead I. The most marked inversion occurs in lead III. These observations indicate that the average direction in which the excitation wave travels over the auricles is upward and to the left rather than downward and to the left as in the normal rhythm. This makes it seem likely that A-V rhythms of type 1, originate in the region of the coronary sinus. In A-V rhythm of type 2 the P-wave occurs simultaneously with R and is invisible in all leads. In A-V rhythm of type 3 the P-wave is inverted in leads II and III. In the single instance in which we have obtained an A-V rhythm of this type in lead I the P-wave was invisible. This was also the case in an A-V rhythm of type 3 described by Williams and James⁸ and in another case described by White.⁹ It seems likely therefore that this is the rule and that the excitation wave does not follow exactly the same course over the auricles in A-V rhythms of type 3 that it follows in those of type 1.

The ventricular complex in A-V rhythm is usually of the normal type since the excitation wave is distributed over the ventricles by the same route as in the

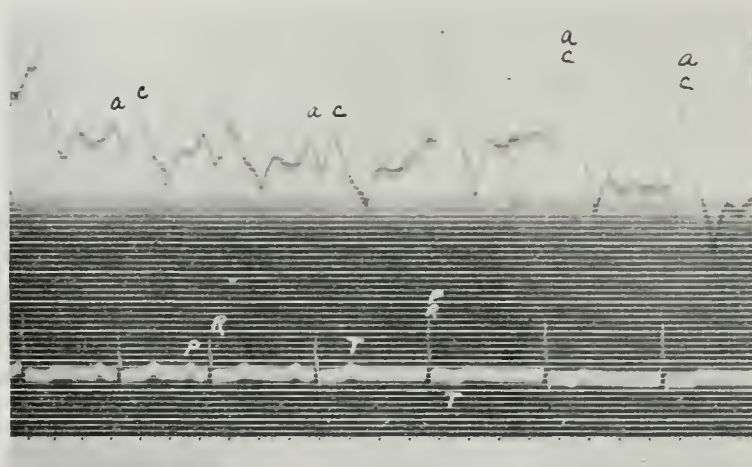


Fig. 6.—Lead III. An A-V rhythm of type 3 from the same patient as Fig. 4. The ventricular complex is abnormal during the A-V rhythm in that R is taller and is not notched and T is inverted.

normal rhythm. Occasionally, however, the ventricular complexes are distinctly abnormal during an A-V rhythm of type 2 or 3. This may occur in normal individuals and is most common in lead III. In Fig. 6, for example, the ventricular complexes of the ectopic rhythm are distinctly different from those of the normal rhythm; the R-wave is taller and is not notched and the T-wave is inverted. Since the origin of the A-V rhythm in this case was well above the bifurcation of the His bundle as is shown by the fact that there is no R-P interval the abnormality of the ventricular complexes can we believe best be explained by assuming that the center which gave rise to the ectopic rhythm was so situated in the A-V node that the excitation wave did not pass through the lower junctional tissues by the normal route and therefore did not pass down both branches of the His bundle simultaneously.

THE IDIOVENTRICULAR RHYTHM OF COMPLETE HEART-BLOCK.

In complete heart-block the stimuli which are formed at the sinus node are prevented from reaching the ventricles and the most rhythmic of the ventricu-

lar centers acts as pacemaker for these heart chambers. The idioventricular rhythm in such cases is a slow one, usually about 30 per minute. Its rate is influenced by exercise but not as a rule by vagus stimulation. The rhythm is a homogenetic one: its failure to be influenced by vagus stimulation is probably due to the destruction or functional depression of the vagus fibers that go to the ventricles by the same agent which produced the heart-block.

The ventricular complexes of the electrocardiogram in complete heart-block is usually of the normal form indicating that the idioventricular pacemaker is situated in the junctional tissues below the block. Occasionally, however, the

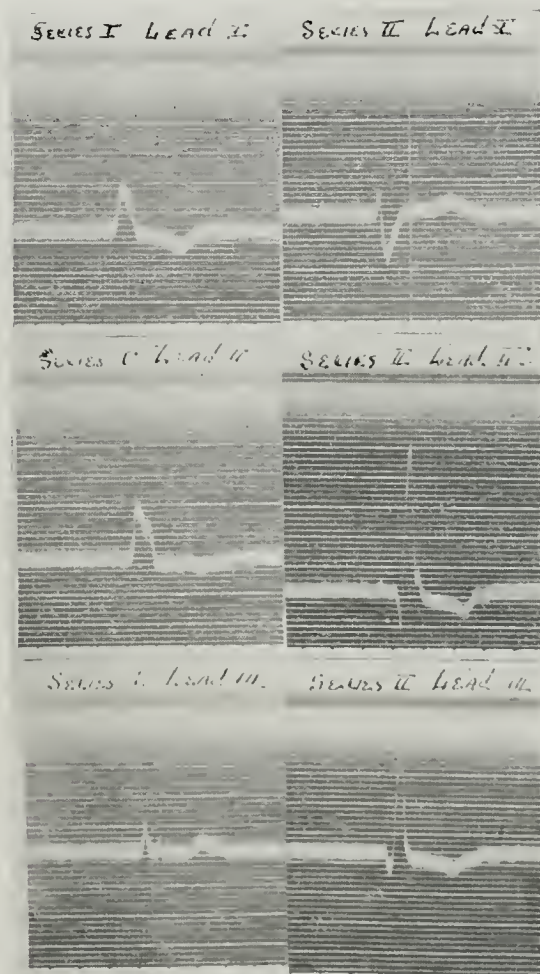


Fig. 7.—Two distinct types of ventricular complex obtained from a case of auricular fibrillation with complete heart-block. The type of ventricular complex labeled Series II was the one usually present. (See discussion in text.)

ventricular complexes are abnormal and such cases must be explained by assuming that the controlling center lies below the bifurcation of the His bundle or that if the center lies above this point there is a block in one branch of the His bundle. Still more rarely the type of ventricular complex in complete heart-block changes from time to time and when this occurs one must assume either that there are two centers competing for the position of ventricular pacemaker and that now one and now the other fills this post or that there is a single center and that the changes in the form of the ventricular complex are due to variations in the conductivity of the branches of the His bundle. It is usually impossible to decide which of these two explanations is the correct one. An ex-

ample of complete heart-block in which two types of ventricular complex were observed is given in Fig. 7.

SUMMARY.

In conclusion we may say that we believe Lewis' division of ectopic rhythms into two groups, homogenetic rhythms and heterogenetic rhythms, is in harmony with the facts and that it is of the greatest importance since it distinguishes between ectopic rhythms which are essentially pathologic and those which are essentially physiologic in origin. The principal differences between the two types of ectopic rhythms are tabulated below.

HOMOGENETIC.

1. Slow rate.
2. No pause at end of abnormal rhythm.
3. Slowed by vagus stimulation.
Rate increased by exercise.
4. No marked nor abrupt change in heart rate at beginning and end of abnormal rhythm.
5. Practically always originate in junctional tissues.
6. Essentially physiologic in origin.

HETEROGENETIC.

1. Very rapid rate.
2. Post-paroxysmal pause.
3. Usually not affected by vagus stimulation or exercise.
4. Abrupt and marked change in heart rate at onset and end of abnormal rhythm.
5. Usually originate in auricular muscle, rarely in junctional tissues.
6. Essentially pathologic in origin.

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THE EFFECT OF VENOUS STASIS ON THE PROTEINS OF HUMAN BLOOD SERUM*

BY ALBERT H. ROWE, M.S., M.D., SAN FRANCISCO, CALIF.

THAT mechanical increase in the blood pressure does not increase the concentration of arterial or venous blood was shown by Böhm¹ and Asher.² On the other hand, it has long been known that venous stasis does increase the concentration of the blood. Grawitz,³ Schultz and Wagner⁴ and many others have studied the increase in the erythrocytes, hemoglobin, specific gravity, and total solids of the whole blood that results from stasis.

The total protein of serum also increases with stasis and the estimation of this increase gives a less erroneous idea of the amount of stasis present than does a determination of the concentration of the whole blood. This fact is shown in many cases of cardiac failure where there may be an increase in the red count and hemoglobin, as well as in the specific gravity and total solids, while the serum is definitely thinner than normal. An explanation of this apparent discrepancy is afforded by the work of Cohnstein and Zuntz⁵ who showed that with a constant number of red cells in the circulatory system, their number in any blood vessel depends on the width of that vessel. Thus narrow capillaries hold relatively much plasma and few red cells, which forces extra reds into other parts of the circulatory system. It can be seen, therefore, that changes in the width of vessels in disease can alter the true relation of red cells to plasma. Moreover Böhme⁶ has shown that the red count of capillary blood is increased above normal with venous stasis while the concentration of the serum is not changed. The same holds true with the red count and the serum when cold is applied to the skin, as was shown by Grawitz,³ Winternitz⁷ and Nonnenmacher.⁸

Kreibich,⁹ while doing refractometric determinations of total protein in the blood sera, noticed accidentally that venous blood which had been under moderate stasis showed a higher refractive index than did arterial blood but he failed to recognize the cause. To Schwenker,¹⁰ working under Böhme,⁶ belongs the credit of working out the fact that the total protein of the blood serum progressively increases with the degree of venous stasis. For the determination of the proteins the refractometric method of Reiss¹¹ was used. Some of his data have been incorporated in articles by Reiss and Böhme together with some of their individual observations on this same problem. Schwenker definitely showed that the longer stasis was sustained the higher was the increase in total protein. Thus he found that in 16 minutes there was an increase from 7.4 per cent protein to 11.4 per cent while in 42 minutes the increase was from 7.2 per cent to 12.16 per cent. His method was to produce stasis with the inflated arm band of a blood pressure machine, the pressure being held stationary throughout the experiment at a point just a little above the diastolic pressure as advised by Zur Verth,¹² in order to give the most intense stasis. A

*From the Medical Service and Chemical Laboratory of the Massachusetts General Hospital, Boston.

lumbar puncture needle with stylet was inserted into the median vein before stasis was begun and was left throughout the experiment. When a specimen of blood was taken the stylet was withdrawn from the needle's bore and reinserted when enough blood was obtained. Schwenker also determined that even the physiological stasis produced by hanging the arm over the bedside, as often occurs in sleep, gave an increase of .7 per cent protein in nine minutes.

Cohnheim's¹³ explanation of this increase in total protein due to venous stasis still holds. His assumption was that a protein-poor fluid passes out

NAME	AGE	DIAGNOSIS	DURATION OF STASIS	DEGREE OF STASIS	ALBUMIN		
					NO STASIS	WITH STASIS	INCREASE DUE TO STASIS
1. G. C.	21	Cardiac neurosis	1 min.	Tourniquet	5.7		
2. H.O.M.	50	Arteriosclerosis Chronic interstitial nephritis	1½ min.	"	5.3	5.6	.3
3. F. G.	50	Fractured femur and fibula	1¾ min.	"	4.4	4.6	.2
4. Z. M.	17	Chronic nephritis	8 min.	75mmHg.	3.85	4.2	.35
5. E. L.	40	Gall stones Arteriosclerosis	10 min.	100 "	4.8	6.1	1.3
6. I. R.	53	Duodenal ulcer	10 min.	100 "	5.	6.2	1.2
7. J. I.	35	Pulmonary tuber- culosis. Hyper- thyroidism	13 min.	90 "	4.6	5.8	1.2
8. J. B.	41	Hysteria	15 min.	100 "	5.	7.6	2.6
9. P. M.	34	Subacute bronchitis	15 min.	100	5.7	7.65	1.95
10. M. S.	40	Constipation	15 min.	100	5.2	7.1	1.9
11. W. B.	43	Dyspepsia	15 min.	100	5.4	7.9	2.5
12. HOM	50	Arteriosclerosis Chronic interstitial nephritis	20 min.	120	5.3	8.2	2.9

The normal values for albumin, globulin and nonprotein as determined by Robertson's method were recently published by Tranter and Rowe.²²

through the capillary walls into the surrounding tissues and finally into the lymph spaces which consequently increases the concentration of the serum in the veins and also the amount of lymph in the lymphatics, both of which effects were shown by Schultz and Wagner to occur with stasis. Water and salt pass in and out of the blood through the capillary walls with the greatest ease. Protein has this same power to a much less extent, which is well demonstrated

in a research by Morawitz¹⁴ in which he showed that after large bleedings in animals the refractive index rose towards the normal value within a few hours, showing that large amounts of protein must have gone through the capillary walls into the blood stream. The same thing is shown by the older work of Magnus¹⁵ who proved that the protein content of the blood underwent definite changes when large amounts of physiological salt solution were injected intravenously.

This increase in the refractive index resulting from stasis is, in a small

GLOBULIN			TOTAL PROTEIN			NONPROTEINS	
NO STASIS	WITH STASIS	INCREASE DUE TO STASIS	NO STASIS	WITH STASIS	INCREASE DUE TO STASIS	NO STASIS	WITH STASIS
2.			7.70	7.87	.17	1.3	1.3
1.9	2.	.1	7.2	7.6	.4	1.3	1.3
2.7	2.8	.1	7.1	7.4	.3	1.1	1.1
2.3	2.8	.5	6.15	7.	.85	1.4	1.4
3.1	3.6	.5	7.9	9.7	1.8	1.1	1.1
2.3	2.6	.3	7.3	8.8	1.5	1.2	1.2
2.5	2.9	.4	7.1	8.7	1.6	1.3	1.3
2.4	3.1	.7	7.4	10.7	3.3	1.2	1.2
2.	3.24	1.24	7.7	10.89	3.19	1.3	1.3
2.	2.6	.6	7.2	9.7	2.5	1.	1.1
2.1	2.7	.6	7.5	10.6	3.1	1.4	1.4
1.9	3.3	1.4	7.2	11.5	4.3	1.3	1.3

but definite measure, due to the increased CO_2 contained in the blood. Koranyi and Bence¹⁶ have found that the refractive index of serum is increased when CO_2 is passed through the whole blood. The cause of this increase was shown by Hamburger¹⁷ to be due to the passage of water and chlorides from the serum into the erythrocytes along with the passage of certain organic substances from the red cells into the serum. Schwenker and Böhm found that in mild stasis the CO_2 increases the refractive index to an extent equal to .1 per cent protein

while in very severe artificially produced stasis the CO_2 may cause an increase equal to from .2 to .42 per cent protein. No definite differences could be made out by these authors between normal venous and capillary blood, showing that the amount of CO_2 that physiological venous blood contains does not influence the refractive index.

The question arises whether the stasis occurring in diseased conditions would make the refractive estimation of total proteins useless. When the venous pressure is higher than normal throughout the whole circulation, as Movitz and Tabora¹⁸ as well as other investigators have shown is the case in heart insufficiency with general edema, the refractive index of venous and arterial blood would differ very little. But when marked edema for example is present in the arm, from a vein in which blood is taken, it is entirely possible that the index will be higher, though only to a slight degree, than that of the unobstructed arterial blood. That this is probable was shown by Achard and Demanche¹⁹ who found that the venous blood taken from an edematous extremity has a higher refractivity than blood taken from the same extremity after the edema has been pressed out, which procedure does away with the stasis caused by the extreme edema. This effect of stasis is not transmitted to the capillary blood except in rare cases since Böhme showed that serum from capillary blood taken from an arm under stasis yields the same refractive index as does the serum from the arterial blood of the same patient. Thus in order to ascertain if edema in the arm, from a vein in which blood is taken, is causing stasis which yields a comparatively large error, it is only necessary to see if the refractivity of the venous serum compares with that of the capillary serum.

The object of the experimental part of this paper has been to show how this increase in protein which occurs with stasis distributes itself in the albumin and globulin fractions and to watch the effect of this stasis on the non-protein elements. T. B. Robertson's²⁰ microrefractometric method has been used in all these determinations. All measurements have been made by the automatic pipette recently described by the author.²¹

To produce the desired stasis in the veins of the forearm, the arm band of a blood pressure machine was placed around the arm and the pressure was sustained at a constant level between the systolic and diastolic pressures. While the stasis was still active, about 10 c.c. of blood were drawn into a dry Luer syringe, which amount yields ample serum for proper controls. An equal amount was then taken from the other arm without causing any stasis. These specimens of blood were then allowed to clot completely and the serum was separated by centrifugalization. Since hemolysis increases the refractive index, no hemolyzed sera were used. The technic of Robertson was followed exactly except that .5 c.c. of serum instead of 1 c.c. was used in the protein precipitation with heat and acetic acid. Normal and pathological cases were used in order to determine if any difference in the partition of the increased protein between the albumin and globulin fractions occurred in either health or disease. The patients were all taken from the Out-Patient Department of the Massachusetts General Hospital. Since exercise increases the refractive index, all patients were allowed to rest quietly from $\frac{1}{2}$ to 1 hour before blood was taken.

From the experimental data given in the accompanying table it appears:

1. That with a constant degree of venous stasis between the diastolic and systolic pressures, the total proteins increase with the duration of the stasis.
2. When blood was taken from a vein, one minute after an ordinary tourniquet was applied to produce stasis, there was only .17 per cent increase in the total protein and therefore too small an amount to split into albumin and globulin.
3. In all of the remaining experiments except the fourth one, the increase in albumin was greater than was the increase of globulin. Part of this difference is probably due to the excess of albumin over globulin than exists in the normal sera of the cases in this series.
4. The nonprotein elements underwent no change due to the stasis.
5. In the fourth case the globulin increase was greater than the albumin possibly due to the fact that the amount of globulin in the normal specimen was greater in relation to the albumin than in any other case of this series.

From this and other experiences with Robertson's method, it appears that certain precautions must be taken in order to get correct results.

1. In obtaining blood from a vein, it is important to draw it off within one-half minute and always within one minute after the application of the tourniquet.
2. The syringe must be dry so as not to dilute the serum obtained.
3. The sera which are hemolyzed cannot be used since the refractive index is increased by hemolysis.
4. The patient must be at rest, preferably reclining, for one-half hour before blood is taken.

Work is now in progress on the effect of hemolysis, diet, and exercise, as well as disease on the relation of albumin to globulin in the blood serum.

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THE PROTEIN POISON OF VAUGHAN*

BY R. W. PRYER, M.S., DETROIT, MICH.

MEDICAL literature is filled at the present time with articles on anaphylaxis or protein sensitization, and the theories concerning this phenomenon are almost as numerous as the workers in this branch of modern medicine. It is not my intention to advance any theory concerning this, and I do not think it necessary to mention any of them at this time, except that of Vaughan,^{8, 23} which is as follows:

Sensitization consists in developing in the animal a specific protelytic ferment, which acts upon the protein bringing it into existence, and no other. That is, if you inject a protein parenterally into an animal, such as the guinea-pig, the cells of this animal immediately set to work to get this protein out of the way. They accomplish this by digesting it by means of a ferment. This ferment in digesting the protein liberates a poison, which, however, is set free so slowly that the animal is not much affected. This ferment is produced rather slowly at first, but after a time the protein is all digested, and an excess of the ferment is stored in the body cells as zymogen. After this has taken place, a second injection of the same protein, and no other, will cause a liberation of this specific ferment and digestion of the protein with an immediate production of large quantities of poison sufficient, if dosage and time intervening between are properly regulated, to cause the death of the animal. This poison can also be prepared by chemical means, and it is with the properties and attempts at purification that this paper deals.

CHEMICAL PRODUCTION OF PROTEIN POISON.

The production of protein poison by chemical means was first reported by Vaughan¹ and Wheeler² in 1905; and their method with few minor variations has been followed in the preparation of the poisons with which I have worked.

A weighed amount of protein is placed in a flask, and shaken with twenty volumes of absolute alcohol containing 2 per cent of sodium hydroxide, in order to thoroughly mix the protein with the solution and avoid caking when the suspension is heated. This mixture is then heated in a water bath, with constant stirring, under reflex condenser at the boiling point of alcohol for one hour after the alcohol begins to boil; temperature of water in the bath being 86° to 92° C. The mixture is allowed to cool and is then filtered, keeping as much of the residue in the flask as possible. After the extract has been filtered off, the small amount of residue on the paper is returned to the flask, and the combined residues extracted with twenty volumes of absolute alcohol as before. In all, three extractions are made. This divides the protein into two portions, one soluble in the alkaline alcohol and very poisonous, but apparently nonspecific; the other insoluble in alcohol and specific. These extracts are combined, filtered if neces-

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sary, and then made slightly acid with hydrochloric acid. This precipitates practically all the sodium as sodium chloride, which is filtered off. The filtrate is evaporated to dryness, preferably in a partial vacuum. The resulting residue is a brownish powder, very hygroscopic and readily soluble in alcohol and water, and is the protein poison. When prepared in this way, the poison is known as the crude poison, and can be purified partially by dissolving again in absolute alcohol and filtering; this removes considerable quantities of sodium chloride. The filtrate from the salt is again evaporated to dryness in partial vacuum and is usually somewhat more toxic than at first. This is the ordinary routine method of preparing the protein poison, and is the one used in preparing most of the poisons that I have studied.

ACTION ON ANIMALS.

No definite chemical test having been found for the identification of this poison, all tests for its presence and amounts are necessarily physiological. Similarly as in experimental work in anaphylaxis, the guinea-pig reacts promptly and is the best of the common laboratory animals for this work. Vaughan in his text-book on "Protein Split Products," pp. 125-6, sums up the action of the poison on guinea-pigs so thoroughly that I can do no better than to repeat his observations here. "When doses of this powder are given intraperitoneally in amounts varying from 8 to 60 milligrams, according as to whether we have been careful to remove most of the common salt or not, a fatal result follows in guinea-pigs in from thirty to sixty minutes. Within fifteen minutes after injection the temperature begins to fall, and sometimes within half an hour has reached 94° F., or even lower. At first, after an interval of from five to ten minutes immediately following the injection, the animal appears restless, runs about the cage, and shows a great tendency to scratch itself, this undoubtedly being due to itching sensations in the skin, caused by irritation of the peripheral nerves. The animal then begins to show evidence of lack of coordination, which is rapidly followed by partial paralysis, which is especially marked in the hind extremities. This stage lasts for from five to ten minutes, during the latter part of which the animal usually lies quietly on one side. From this stage the animal passes into what one might term the convulsive stage. These convulsions are usually clonic in nature, and, as a rule, at first involve only the neck muscles, the head being momentarily drawn backward on the back. At first these convulsions are but slight in degree, and are separated by considerable intervals of time. Soon, however, they become much more frequent and of much greater severity. Gradually they become more and more general in their extent, until all the muscles in the body become involved in violent clonic convulsions. This stage when present presages a fatal outcome; rarely an animal recovers after reaching the convulsive stage. During a convulsion or occasionally in the interval of calm, respiration ceases. The heart, however, continues to beat, at first with perfect regularity and no acceleration; indeed, the rate seems to be somewhat slower than normal. Gradually the beat becomes more and more feeble, the rate and regularity being preserved to the end. It is usually only after an interval of from three to four minutes after the cessation of respiration that the heart

ceases to beat. As has been previously stated, a fatal issue, if it occurs at all, always results within one hour after injection, and usually within thirty to forty minutes. This is to a large extent independent of whether the dose is the minimum lethal dose or two or three times that amount. It is certainly entirely independent of the size of the pig.

"At autopsy no special gross lesions can be made out. The peritoneum is smooth and shiny throughout, and there is not the slightest evidence of either hemorrhage or even marked congestion in the omentum or mesentery."

The poison is acid in reaction (from 3 to 6 per cent acid calculated as hydrochloric acid), but this is only partly due to hydrochloric, since an aqueous solution of poison shaken on a machine with silver carbonate showed but a slight decrease in acidity after filtering, and no decrease in its toxicity. Fifty cubic centimeters of a water solution of which one cubic centimeter equaled 50 milligrams or a minimum lethal dose of the poison when given intraperitoneally to a guinea-pig, and with an acidity of 5.1 per cent calculated as hydrochloric acid was shaken for three hours with three grams of finely powdered silver carbonate. After filtering, the filtrate still killed in one cubic centimeter doses and showed an acidity of 4.7 per cent.

ACTION OF IMMISCIBLE SOLVENTS.

Ether removes a slight amount of waxy resinous material from acid solution of the poison or from solutions made slightly alkaline with either the fixed alkalies or ammonia. The material removed is practically nontoxic in either case. Chloroform, petroleum ether or benzine also remove small amounts of nontoxic material, as will amyl alcohol. An excess of acetone added to aqueous solutions of the poison precipitates a slight amount of nontoxic material. From the dry powdered poison contained in the thimble of a Soxhlet extraction apparatus, chloroform removes slowly, part of the poison, but the amount of this extract, after evaporation of the chloroform, necessary to kill is as much or more than the original poison.

HEAVY METALS.

Many of the salts of the heavy metals give abundant precipitates with the protein poison. Some of the better ones for this purpose are the chlorides of mercury, copper, and platinum. Mercuric chloride precipitates only a small part of the poison from aqueous solutions. From solutions of the poison in absolute alcohol, mercuric chloride in absolute alcohol precipitates the greater part of the poison as an amorphous flocculent precipitate. On removal of the mercury from this precipitate by means of hydrogen sulphide, the residue after filtering and evaporation of filtrate in partial vacuum is much more toxic than before. If this residue is again dissolved in absolute alcohol and reprecipitated by mercury as before, the precipitate suspended in alcohol and mercury removed by hydrogen sulphide and filtrate evaporated as before, usually gives a still more toxic residue. I have been able in this way to obtain poison that would kill guinea-pigs in doses of .0005 grams, when given either intravenously or intracardially. But this is as far as I have been able to carry the purification by

this means. Usually when I get about this far, the toxicity begins to decrease, and continual reprecipitation by means of alcoholic mercuric chloride will not carry the purification any farther. All attempts to crystallize this mercuric precipitate have failed, and so, while I concede that this is one of the most efficient means of purification of which I know, it is a failure as a means of complete purification.

Copper chloride in an aqueous solution of poison gives no precipitate; however, in solution in absolute alcohol it will, providing the solution is not too concentrated. This precipitate, after removal of copper by hydrogen sulphide, is found to be very toxic, and I have been able to obtain in this way poisons that kill guinea-pigs in .0005 gram doses when injected intravenously. However, this precipitate, either before or after removal of the copper, is amorphous and cannot be crystallized. It was found impossible to determine the chemical composition of the poison in this way.

Platinic chloride precipitates part of the poison from either aqueous or alcoholic solution, but in either case before or after the removal of the platinum, the poison cannot be crystallized.

Although the most potent poisons have been obtained by precipitation with the heavy metals mentioned, the nature of the poison could not be determined. The precipitates of the poison with mercury and platinum in alcoholic solution are insoluble in any of the common solvents. The copper precipitate is nearly insoluble in alcohol, is absolutely insoluble in the other common solvents except water, which dissolves it readily. Attempts were made to purify this copper precipitate by dialysis, but were unsuccessful, as were all other attempts to crystallize it.

Dialysis of the free poison in aqueous solution shows that the poison diffuses slowly through collodium membranes. However, the time required for dialysis is so long that the poison obtained in this way is no purer than the original, so this cannot be used as a means of successful purification.

β -IMIDAZOL-ETHYLAMINE.

Dale and Laidlaw¹⁷ in their work on the physiological action of the compound obtained from histidine by removal of carbon dioxide and known as β -imidazol-ethylamine (this compound will be referred to as β -I.) noted the similarity of the action of this compound to anaphylactic shock, which is similar to if not identical with the action of the protein poison.

There are indeed many points of similarity between the action of β -I. and the protein poison. For example, both cause contraction of the bronchioles, and death results from asphyxia. Both lower blood pressure in dogs.^{11, 12} The fatal dose of β -I. for the guinea-pig when injected intravenously is .0005 gram, which is the same as required of our purest poison. Another striking point of similarity is the fact that a nonlethal dose of either will protect an animal against a lethal dose of the same given shortly afterwards. However, points of difference are practically as numerous as points of similarity. Protein poison is slowly destroyed by heat, particularly if any alkali or mineral acid is present, while β -I. is not. Indeed, one of the final steps in its preparation consists in heating with

concentrated hydrochloric acid.^{15, 20} Protein poison can be removed from aqueous solution by simple salting-out processes, as I shall show later. In order to fully satisfy myself on this point, I have many times tried to separate the compound β -I., histamine, or ergamine, as it is sometimes called, and have always failed to do so.

The method used was that of Kossel and Kutscher,^{24, 25} and is the same as Barger and Dale¹⁸ used in the separation of β -I. from the intestinal mucosa, and Ackerman,¹⁶ in its separation from bacterial cultures. I shall describe later a slight modification of the method of preparing protein poison, whereby I have been enabled to obtain sufficient poison from ten grams of casein to kill 8,000 guinea-pigs when given intravenously. Calculating the fatal dose of β -I. intravenously as .0005 gms., this would mean, if β -I. were the active principle of protein poison, that casein contains over 40 per cent of histidine. It seems that this at once precludes any possibility of β -I. being the protein poison, since casein contains less than 3 per cent of this amino acid.²⁴

The possibility of the poison being a choline derivative has been considered, since atropin protects against either, but I have been unable to find any trace of neurine or similar compounds by any of the methods ordinarily used, and heating with baryta water soon destroys the protein poison, whereas it is one of the steps in the isolation of the choline derivatives.²²

AMINO ACIDS AND THEIR PRODUCTS.

The end-products of protein hydrolysis are the amino acids, and the extraction of proteins by means of 2 per cent sodium hydroxide in absolute alcohol is one method of hydrolysis. A study of the amino acids obtained from protein poison was reported by Wheeler in 1909,²⁶ but did not throw much if any light on the chemical constitution of the poison. With the idea in mind that perhaps some of the amino acids either before or after treatment with the alkaline alcohol might be poisonous, the following experiments were carried out:

One hundred milligrams of leucin were dissolved in water and injected intraperitoneally into a guinea-pig. The animal was unaffected. One hundred milligrams of leucin were heated on a water bath with 300 c.c. of alkaline alcohol for three hours, filtered, and the filtrate made acid with hydrochloric acid. The sodium chloride was filtered off, and the filtrate was evaporated to dryness, leaving 75 mgs. of residue. This residue was dissolved in water and injected intraperitoneally into a guinea-pig with no noticeable reaction. Glycocoll, alanin, histidine, tyrosin, phenylalanin, glutaminic acid, aspartic acid and tryptophane were tested in the same way and gave negative results. Many different mixtures of amino acids were tried, and also the products of these mixtures after treatment with alkaline alcohol, and in no case was any appreciable quantity of poison formed.

As an example of some of the mixtures that were tested in this way, the following experiment is recorded:

Fifty milligrams of each of the following were mixed intimately in a mortar: alanin, phenylalanin, leucin, glycocoll, tryptophane, glutaminic acid, histidine, tyrosin, uric acid, and glucose. This mixture was then extracted in the usual way with three successive portions of 250 c.c. of absolute alcohol containing 2 per cent of sodium hydroxide. The extracts were combined, filtered, made slightly acid with hydrochloric acid and filtered. The filtrate was evaporated to dryness and the weight of residue found to be 370 mgs. One-half of this was dissolved in water and injected intraperitoneally into a guinea-pig, producing no reaction.

ACTION OF VARIOUS GASES.

Carbon dioxide, oxygen, hydrogen or air do not appreciably affect the toxicity of the protein poison when passed through aqueous solutions, but hydrogen sulphide slowly lessens the toxicity.

Ozone gas prepared electrolytically from air and passed through aqueous solutions of poison at such a rate that each cubic meter of air contains 0.2 gms. of ozone slowly weaken the poison. It was found to be rather a difficult matter to treat solutions of the poison in this way, on account of the very energetic frothing that occurred. It was only by using a large number of bulbs, one above the other, somewhat on the plan of a reflux condenser about five feet high, and aspirating the ozonized air through by means of a water pump, that these solutions could be ozonized at all, and several days were required to complete the operation. It was found that it was possible to so ozonize solutions of the poison that they would still be poisonous, although the toxicity was decreased, and they would no longer give the Million reaction. The biuret test still persisted, however, and the question of whether or not the poison was a protein could not be settled in this way. The limits between the destruction of the ability to give the Million test and the complete destruction of the toxicity were very narrow. During the first two years of this work on protein poison, Witte's peptone was the protein usually worked with, and an attempt was made to ozonize this protein before extraction with the alkaline alcohol. For this purpose 100 gms. of Witte's peptone were dissolved in a liter of water and ozonized for a period extending over nearly four months. It should be stated, however, that the machine used to generate the ozone was continually getting out of order and required careful attention and frequent repair. The actual number of hours that the ozone was aspirated through this solution was 239. At the end of that time the solution was washed out of the apparatus, evaporated to dryness on the water bath and powdered; yield, 80 grams. This powder still gave a beautiful biuret test, but the Million was very faint, if given at all. It was soon found that this ozonized protein, which was very dark in color and possessed a very disagreeable odor, would no longer produce a protein poison when split up in the usual way.

YIELD OF POISON.

The amount of poison obtained from different proteins varies considerably. The germ substances of Vaughan, such as tubercle, typhoid, colon, and others, yield only about 30 to 40 per cent of poison by weight. With casein I have been able to obtain 90 per cent, and with peptone from 50 to 75 per cent. Of all the steps in the preparation of protein poison, the most important is that of neutralization. If the combined extracts are neutralized to phenolphthalein, but little poison is obtained, and enormous amounts are required to produce death. It has been my experience that if the alkaline alcoholic solution is made one per cent or more acid to phenolphthalein by means of hydrochloric acid, and the precipitated salt well washed with absolute alcohol, the largest yield of toxic substance is obtained. Another slight modification in technic that I have introduced in the preparation of this poison consists in not evaporating the acid al-

colhic solution to complete dryness and powdering it, but in evaporating under reduced pressure till the alcohol is all distilled off, and then taking up in water. Then of course if one desires to know the amount by weight, evaporate a measured portion to dryness at 100° C., cool in a desiccator and weigh. This obviates the necessity of long heating of the poison and produces a much more potent poison. In this way I have been able to prepare sufficient poison from ten grams of casein to kill 8,000 guinea-pigs when injected intravenously. There seem to be slight differences between the poison prepared in this way and the one prepared in the usual way. The most striking difference lies in the ratio of the fatal dose for the rabbit as compared to that for the guinea-pig. With the poison as ordinarily prepared, the ratio of dose for these two animals is practically proportional to body weight, usually about eight to ten times as much being required for ordinary rabbits as for guinea-pigs, when injected by the same route. With the poison prepared in this way, the amount necessary to kill rabbits intravenously is about seven hundred times as much as for guinea-pigs.

SOME SERUM REACTIONS.

Another remarkable thing is that if, after a rabbit dies following an intravenous injection of the poison and as much serum as possible is obtained, this serum is nontoxic fresh. However, after one hour in the incubator it becomes toxic for the guinea-pig; one cubic centimeter being sufficient to kill when injected intravenously, while the same amount is nontoxic if injected after fifteen, thirty, or forty-five minutes in the incubator.

On the other hand, if a nonfatal dose be injected into a rabbit and shortly after the rabbit be bled and the serum obtained, this serum is nontoxic either before or after incubation.

Normal rabbit's serum mixed with a solution of poison so that less than a fatal dose for the guinea-pig when given intravenously is contained in each cubic centimeter when placed in the incubator becomes toxic in about one hour. One cubic centimeter samples taken out every ten minutes and injected intravenously into guinea-pigs are nontoxic after ten, twenty, thirty, forty, or fifty minutes, but kill promptly after one hour.

Normal guinea-pig serum when treated in the same way becomes toxic after twenty minutes in the incubator, and the toxicity disappears after forty minutes.

ACTION OF ALKALIES AND ACIDS.

Barium hydrate added to aqueous solutions of protein poison precipitates it completely. This precipitate is with difficulty soluble in water or physiological salt solution, but is readily soluble in alcohol or weakly acidified water. It is also readily soluble in barium hydrate solution and evidently forms a barium salt, since this new compound is readily soluble in water but insoluble in alcohol. The toxicity of the poison is weakened by contact with alkali and is soon destroyed by heating the barium compound.

A few drops of either acid or alkali added to an aqueous solution of the poison precipitate it as a gummy, resinous mass, and the toxicity decreases rapidly, particularly if heat is applied.

SALTING-OUT METHODS.

One of the best methods of purification that I have been able to discover consists in saturating the aqueous solution with sodium chloride. This precipitate is then dissolved in absolute alcohol to free it from excess of salt, filtered and evaporated. One very peculiar thing about this method is the fact that not alone is the poison increased in toxicity,—that is, a smaller amount is required to kill than before, but actually a greater number of fatal doses is obtained. For example, starting with 200 fatal doses for the guinea-pig when given intravenously, I have been able by precipitation in this way to obtain sufficient poison to kill over 300 guinea-pigs when given in the same way. This cannot be carried further, however, and reprecipitation of this by saturation with salt, while it never decreases the toxicity, fails to increase it as the first precipitation does. Just why a greater number of fatal doses can be obtained in this way, I am unable to say. I have failed to show that the filtrate from saturated salt solution has any marked protective action against the minimum lethal dose of the poison injected at the same time or subsequently to the poison injection. This filtrate contains slight amounts of poison and consequently if injected previously to the poison gives a slight protection for a few hours. However, the poison always shows this property. Many attempts were made to obtain a higher degree of purification of the poison by using varying concentrations of salt, but were unsuccessful.

As has been reported before,²⁶ magnesium sulphate and ammonium sulphate will also precipitate the greater part of the poison. Magnesium sulphate is not as effective as sodium chloride; and because of the solubility of ammonium sulphate in alcohol, this salt is not a good one to use, because if one attempts to remove it by dialysis, part of the poison is lost.

CONCLUSIONS.

1. All proteins contain a poisonous group.
2. The protein poison is not β -imidazol-ethylamine, although the physiological actions of the two are very similar.
3. The protein poison is not a choline derivative.
4. Protein poison is not a simple chemical compound, but is a protein change product, acid in reaction, capable of forming salts and reacting much like the globulins in its behavior to neutral salts.

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THE TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM*

BY HARRY G. PAMMENT, M.D., TOLEDO, OHIO.

THE series of cases covered by this paper consists of 11 cases of tabes dorsalis; 1 of paresis; 1 of tabo-paresis and 1 of a psychosis of syphilitic origin thought by some to be early paresis.

The highest number of injections I succeeded in giving to a single patient was seven and the least was two. The average being four.

Eight cases of tabes and the early paresis were treated with the mercuralized serum as described by Byrnes.¹ One case of tabes was treated with serum alone while using the inunctions of mercury and one with the neosalvarsanized serum. The paretic was treated with neosalvarson directly into the spinal canal using as a diluent the spinal fluid and the tabo-paretic with serum alone while using the inunctions.

After January 1, 1915, I had to use the mercuralized serum entirely because of the difficulty of obtaining the salvarsan and can see no reason why this treatment should not be used while the supply of the latter is so limited.

*From the laboratory department of the Lucas County Hospital, Service of Dr. Louis Miller.

¹Byrnes, C. M.: *Jour. Amer. Med. Assn.*, 1914, lxiii, 2182.

In all cases a Wassermann test was done on the remaining blood serum and a careful analysis of the spinal fluid after each injection. In this way all clinical improvement could be checked up with the changes in the spinal secretion.

The first thing that I noticed in using the mercurialized serum was what I called a severe reaction. The patients invariably complained of severe cramps in the legs, headache and nausea accompanied by a temperature of about 101°. It made me think that I had not prepared the serum properly and the internes were rather inclined to think that I was not warranted in continuing the treatments. However I did continue and soon discovered that in most cases the severe discomfort could be eliminated by heroin 1/12 gr. hypodermic at the time of the injection and a second one ten hours later if necessary. I also used gr. v each of pyramidon and aspirin every three hours for a day or two. This part of the treatment I soon learned to be most important if I wished the patient to continue the treatments. My early cases refused more than two injections because of the severe suffering so I was most happy to find a method for their relief.

The following case reports give the clinical and laboratory findings together with a few facts of interest from the history.

Case No. 1.—Mrs. A. Age 40. Her chief complaint consisted of shooting pains and numbness in the legs and inability to walk well. She received two injections intradurally of 1/50 gr. of the bichloride and as the shooting pains and difficulty in walking improved so rapidly she left the hospital refusing further treatment. I saw this patient four months later when she stated that she had not improved any further and as she was not getting worse she chose not to take more treatments. The cell count on her spinal fluid decreased from 120 to 66 while all the other tests remained strongly positive.

Case No. 2.—E. F. Age 27. His chief complaint consisted of severe pain in the stomach and legs and inability to walk well. He had an operation for gastric ulcer 8 months previously with no relief. This patient was hard to handle and left the hospital after two treatments with no clinical improvement. All the tests on the spinal fluid remaining strongly positive.

Case No. 3.—Mrs. C. Age 42. Her chief complaint is of a continuous pain in her left arm and shoulder of one year's duration. This could not be accounted for except by the positive sign of tabes and the spinal treatments were given hoping to relieve it. She received three injections of 1/50 gr. of bichloride in serum intradurally with no change except that the cells in the spinal fluid dropped from 40 to 15. The reaction was most severe, temperature 102° and what was most distressing an uncontrollable nausea and vomiting that lasted a week each time. Accordingly she refused further treatments and left the hospital about April 1, 1915. The neuritis was unimproved. About three months later she returned to the hospital with a fracture of the humerus in the arm opposite to the one with the neuritis. Examination at this time showed no change in her condition.

Case No. 4.—G. D. Age 41. His chief complaint was due to a gummatous ulceration of the hard palate but he was given three intradural injections of the bichloride because he had all the signs of tabes although the latter was not giving him any trouble. He was given two doses of neosalvarsan and mercurial inunctions also. There were no changes in the tests on the spinal fluid or blood and at the end of two months as the gumma was entirely healed the patient ran away.

Case No. 5.—Mrs. M. F. Age 55. The chief complaint in her case was—pain in the legs and back, incontinence of urine and inability to walk. She was given four injections on general principles although the case was considered hopeless. The fifth treatment was refused because the pain in her legs was made worse but she volunteered the information that the numbness was much better. The spinal fluid was unchanged as was also her condition when I saw her four months later.

Case No. 6.—M. W. Age 54. His chief complaint consisted in paroxysmal pains over the heart and indefinite pain in the legs and back. He received five injections of the bichloride. The cell count decreased from 75 to 28 and the gold chloride reaction had improved, but the Wassermann remained strongly positive. Clinically he was better but refused further treatments because of the severe reaction.

Case No. 7.—Mrs. A. W. Age 38. She had been troubled with failing eyesight and inability to walk well for two years. This patient received six injections of the bichloride intradurally, 1.8 gm. of neosalvarsan intravenously, mercurial inunctions and potassium iodide for a period of four months at the hospital. The only improvement in the spinal fluid was a decrease in the cell count from 71 to 12. The Wassermann on the blood and spinal fluid remaining strongly positive. The clinical improvement was quite marked, both the eyesight and ataxia being much better. This patient was seen two months after the last treatment was given—her condition at this time being about the same as it was when she left the hospital.

Case No. 8.—B. E. Age 46. His chief complaint is as follows: pain and numbness in the legs, incontinence of urine, tight feeling about the chest, cough and expectoration. He received six intradural injections of the bichloride and although he had a severe reaction each time he continued the treatment because he knew he was getting better. After he had had four treatments he had a pulmonary hemorrhage from his tuberculosis and was allowed to go without a treatment for two months. He then received the last two treatments a month apart. The analysis of the spinal fluid and blood at the last treatment showed a cell count of 10 with all the other tests still strongly positive. This patient has been under observation for about eight months and has had the routine treatment with Ung. hydrargyrum. Clinically there was great improvement.

Case No. 9.—P. W. Age 37. His trouble consisted of great weakness, pain and numbness in his legs, incontinence and failing eyesight. He had the primary lesion but three years before. After seven injections of the bichloride the cell count dropped from 200 to 28, the Wassermann became negative 6 weeks before the Noguchi and the gold chloride was greatly improved. Clinically the patient was a well man and worked about the hospital as orderly during the last month.

Case No. 10.—L. H. Age 42. This man was diagnosed as a syphilitic psychosis or possibly a beginning paresis and was troubled with headache, nervousness and an unexplainable desire to run his automobile into things. He admits having had a chancre 20 years before. His pupils are unequal but react to light and accommodation, knee jerks are lively, superficial lymph glands are palpable, the blood Wassermann faintly positive and all the tests on the spinal fluid are strongly positive for a syphilitic infection. The treatment here consisted of six intradural injections of the bichloride at intervals of two weeks, potassium iodide in increasing doses and daily inunctions of mercurial ointment. At the end of two months of treatment the blood Wassermann was negative and at the end of the series of injections the cell count had dropped from 40 to 14, the Wassermann had become negative, the gold chloride greatly improved and the globulin test but faintly positive.

Clinically there was great improvement in the mental condition of the patient who left the hospital soon after the last treatment and has not been heard of since.

Case No. 11.—Mrs. B. G. Age 45. Her complaint consisted in numbness of the legs and perineum, inability to walk well and slight incontinence of the urine and feces as well as cough and expectoration. A diagnostic spinal puncture showed a fluid under pressure with 20 cell, a positive globulin and Wassermann and a change in the gold chloride in the tabes region. The blood Wassermann was negative. The sputum contained many tubercle bacilli. She was given three intradural injections of neosalvarsanized serum and the spinal fluid became normal except for a positive Wassermann. The treatment was discontinued because of a pulmonary hemorrhage. After two months she was given the fourth and final treatment at which time I was surprised to find that the tests on the spinal fluid were all stronger than ever.

Clinically there was great improvement in her tabes and she refused further treatment. This case illustrates that the Swift-Ellis method causes the spinal fluid to approach normal rapidly, but two months without treatment allowed it to go back to where it was when I began the injections.

Case No. 12.—J. L. Age 64. The diagnosis here was paresis and emphysema and

the spinal fluid gave strongly positive tests for syphilis with 24 cells. Three injections of 3 mg. of neosalvarsan in patient's spinal fluid were given two weeks apart and as the patient gradually got worse the treatment was discontinued. At the time of the third treatment the cells had increased to 90 while the other tests on the fluid were still strongly positive. Two months after the last treatment the spinal fluid was again examined. No change was noted except that the cells had dropped to 14.

Clinically there was no improvement—in fact the patient seemed to be worse after each treatment.

Case No. 13.—J. N. Age 47. His complaint consisted of weakness and shooting pains in the legs and as his blood Wassermann was strongly positive he was put on daily inunctions of mercury. After 10 days of this treatment he received 12 c.c. of his own serum in 18 c.c. salt solution intradurally. A second injection of 15 c.c. of serum and 15 c.c. salt solution was given in 16 days. A third and last injection was given in 18 days. At the end of this time the cells had decreased from 58 to 20, the gold chloride had improved, the globulin and Wassermann remaining strongly positive. Clinically the pains had all left and the patient felt so well that he refused further treatments. He worked about the hospital for two months as orderly without a relapse and then left to be married.

Case No. 14.—C. S. Age 49. This patient had attacks of paralysis, an Argyll-Robinson pupil and exaggerated deep reflexes and was diagnosed paresis. She received four injections of increasing amounts of her own serum intradurally and daily inunctions of mercury. The cells fell to normal, the globulin was faintly positive, the Wassermann became negative and the gold chloride remained only slightly positive. The changes in the spinal fluid and the clinical improvement were so marked that I have always felt a little doubtful of this case.

In analyzing the cases it will be seen that those treated with the bichloride are arranged according to the number of treatments given. The greatest clinical improvement was seen in those receiving four injections or more.

Cases Nos. 1 and 2 receiving two treatments. No. 1 showing considerable and No. 2 no improvement whatsoever. Neither spinal fluid showed improvement.

Cases Nos. 3 and 4 received three treatments. No. 3 showing no change in the brachial neuritis, for which the treatments were given, while No. 4 believed himself completely cured but his feeling of well being came from the healing of the gumma. The spinal fluid was practically unchanged.

Case No. 5 received four treatments with no prominent clinical improvement nor important changes in the spinal fluid.

Case No. 6 received five treatments with some clinical improvement and a slight improvement in the spinal fluid.

Cases Nos. 7 and 8 received six injections. Both showed a great clinical improvement and considerable change in the spinal fluid.

Cases Nos. 9 and 10 showed the greatest clinical improvement and these were the only two that I succeeded in getting a negative Wassermann and Noguchi on the spinal fluid.

Cases Nos. 11 and 12 were started early last fall before the article by Byrnes was published and were continued as such for comparison.

Cases Nos. 13 and 14 received three and four injections respectively of their own serum after they had been using inunctions for about 10 days. Both cases showed considerable clinical improvement and some slight changes in the spinal fluid.

It had occurred to me that this latter method might be an efficient way of getting mercury into the subarachnoid space in combination with the specific

antibodies but Swift² recently has pointed out that remarkable changes can be produced by normal serum in some cases.

Although there is nothing startling in the results of this series there was enough clinical improvement to encourage me to continue the treatments and to conclude that patients with syphilis of the central nervous system should be given the benefit of some sort of intradural therapy. However when such good results can be produced with the Swift-Ellis method as reported recently by Smith³ both clinically and in the spinal fluid it should be the method of choice. So long as the expensive salvarsan and neosalvarsan cannot be obtained then the mercurialized serum is certainly indicated.

HETEROPLASTIC BONE AND BONE-MARROW FORMATION ASSOCIATED WITH TUBERCULOSIS IN THE ADRENAL*

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

THERE are three hypotheses which are used in accounting for heteroplastic bone formation. One of these postulates the occurrence of embryonic "rests" of osteogenic tissue, misplaced during development, which in later years, under the stimulus of changed physiologic conditions, commence to grow and to produce bone. Another postulates the transference of osteoblastic cells by way of the blood stream by means of which they arrive in unusual situations where they lodge (embolism) and grow, producing bone. The third making use of the doctrine of metaplasia, holds that any cell may, within certain tentative conditions, change in its morphologic features, and become physically and perhaps chemically similar to other cells arising from the same general layer. To quote Adami—"while there may be conversion of one epiblastic or hypoblastic tissue into another epiblastic or hypoblastic tissue, and of one mesoblastic form of cell into another mesoblastic form, this conversion is of a limited extent; metaplasia of mesoblastic tissue into epiblastic or hypoblastic and vice versa does not occur."

The doctrine of metastasis and of metaplasia are the dominant ones. Of that which postulates embryonic rests there is no proof, save perhaps in the rarest instances and in teratomata. There is no evidence that such remains play any part in the production of heteroplastic bone in any of the usual cases. We believe that it may be practically discarded from this discussion.

The conception of metaplasia is the dominant one, combated though it be by certain pathologists such as Ribbert, who, though he admits physiologic metaplasia, nevertheless denies, at least to a very great extent, its occurrence under pathologic conditions. While we admit that there are a few, comparatively few, cases in which we can see no other explanation for the phenomenon than that held in the doctrine of metastasis (illustrated best by Le Count's case of Meta-

²Swift, H.: Jour. Amer. Med. Assn., 1915, lxv, 209.

³Smith, L.: Jour. Amer. Med. Assn., 1915, lxiv, 1563.

*From the Pathologic Institute of the Cincinnati General Hospital, and the Mary M. Emery Department of Pathology of the University of Cincinnati.

static Osteomata), it seems to us that the logical explanation of practically all osseous heteroplasia can be found in the doctrine of metaplasia. Metaplasia, it seems, being a physiologic process of the widest possible application, must be also a phenomenon occurring under pathologic conditions. The tissues of the body are formed by gradually changing steps that appear in response to physical and chemical conditions. There is no intrinsic embryologic difference between the cells which are to form fibrous tissues and those which are to produce bone. The variations which arise are the results of physical and chemical differences in the environment of the cells which make it necessary that different structures be produced, and of the two orders of environmental factors, the chemical is more important. A fracture heals by fibrous union, not because there is no need for bone, nor because the stresses which might better be withstood by bone are absent, but because the chemical conditions, which demand bone, are absent.

If the proper nutritive conditions are present then only is bone formed, otherwise fibrous tissue of one or another order appears. In other words, there is no sharp boundary line between the various supporting tissues of mesoblastic origin, a fact which is best exemplified in the embryo during development.

How the necessary chemical conditions favoring osseous metaplasia are brought about, we do not know. My friend, Professor E. R. Le Count, has suggested that the presence of free blood plays a part, in what way I do not know. It is, however, an interesting fact that heteroplastic bone is most frequently associated with trauma and with inflammatory changes which are in turn associated with hemorrhage. It is possible that the presence of red blood cells which are colloids of a certain concentration and composition, furnish the proper physico-chemical basis for deposition of salts in concentrations such that the mesoblastic cells during the growth associated with organization are impelled in the direction of bone formation rather than of fibrous tissue production.

The relationship between blood supply and bone formation has been commented upon by various investigators who have observed this type of metaplasia following ligation of the blood vessels of the kidney in which organ there seems a special unknown reason for the appearance of the process. In three out of four cases, after ligation of the renal vessels one may observe bone and medulla formation, and in the affected areas one sees practically the same process at work which takes place during normal ossification of membranes during development. (Sacerdotti and Frattin.) It has also been shown (Poscharissky) that in other organs than the kidney (liver, spleen, ovary, etc.) ligation is not followed by ossification, but only by necrosis and calcification. It seems as though there were an organ disposition to ossification. Poscharissky believes that the differences are due to different circulatory conditions, or to the fact that in some organs a longer time must elapse before ossification occurs. It is our experience that white infarcts of the kidney never show calcification. It is also our experience that mild degrees of calcification (i. e., deposition of small amounts of lime salts as granules in the renal cells) are not infrequent. Tying the efferent vessels of the kidney produces a condition of passive congestion, which is similar to red infarction, in which the red cells pass out into the tissues in large numbers, a condition quite unlike that found in white infarction. There seems

then to be at least a superficial relation between red blood cells and ossification in the kidney.

In the case which I wish to report, there was no evidence of hemorrhage. There was, however, an inflammatory process of a chronic nature which may or may not have been associated with emigration of red blood cells.

The patient, J. H., age 42, a white male, was admitted to the Neurologic Service of the Cincinnati General Hospital on March 13, 1915, complaining that he could not walk. He gave a history of malaria; and of smallpox, but of no other serious illnesses. For 20 years he had had an ulcer upon the right leg. For a number of weeks he had suffered from urinary incontinence.

The present illness began a few days before entrance to the hospital. One evening, he said, his legs began to feel strange. The next morning he had difficulty in walking to his work and finally was helped. He said his legs did not feel like legs and that they were weak. By the time he returned from work he was no longer able to walk.

Physical examination (according to the clinical history, which was obviously incomplete) showed "a well developed and nourished white man. Heart and lungs negative. Abdomen negative. Pupils and movements of eyes, negative. Muscles of the face, tongue and arms negative. Leg-knee jerks increased in both. Double Babinski. No ankle clonus. Legs are useless, but draw up involuntarily and then stay until drawn down. An ulcer nearly encircles (except at back) the right leg from the ankle nearly to the knee. Feces and urine passed involuntarily and frequently." Death occurred on April 11, 1915.

The clinical diagnosis was spastic paraplegia.

The postmortem was done 12 hours after death. The following is a copy of the protocol.

The body was that of a fairly well nourished white adult man 5 feet, 5½ inches long. The pupils were both somewhat dilated, the right more than the left. The skin of the face had a yellowish-brown tinge somewhat like that of Addison's disease. The conjunctivæ were pale. The eyes were sunken. On the inner side of the elbow joint (right) there were puncture wounds of needles. The chest was remarkably barrel-shaped, beside which there was a dorsal scoliosis. The abdomen was navicular. Age apparently 40.

Postmortem lividity was well marked. Rigor mortis was present and more marked in the arms and hands than in the feet. The right knee was slightly flexed. Midway between the sternal and axillary lines and 3 cm. below the intermammary line, was a gaping wound which, except for the fact that its base was apparently (to the touch) cartilage, looked like the wound of an unhealed costectomy. The edges of this seemed clean and healthy, and they were not undermined. The base was covered with a thin sero-purulent fluid. No sinus could be appreciated. On section it appeared that this lesion was one due to a destructive process in the 5th costal cartilage which had also involved the adjacent intercostal tissues. It did not involve the pleura. Eighteen cm. below the right knee and extending toward the ankle (12 cm. anteriorly, and ½ cm. posteriorly) was an ulcerated surface which extended completely around the leg. The margins of this were more or less irregular but were smooth and clean and showed every indication of indolence. The skin immediately about the edges was thickened, white and opalescent. Beyond this, both above and below, the skin and subcutaneous tissues were thickened and edematous, and the skin was pigmented. Such veins as could be seen above the ulcer were dilated and tortuous (varicose). The surface of the ulcer was smooth, red, moist, clean and indolent. The anterior lines of the tibiæ were smooth. There was no edema of the left foot or ankle. The penis and scrotum were cyanotic. Just to the right of the midline of the perineum, at a point where perineum and scrotum

joined, was the circular opening of a sinus which ran anteriorly under the skin of the scrotum. There was no evidence after dissection that this connected with the bladder or urethra, or with any other organ. On the back, scattered here and there, were very many irregular, some almost serpiginous, ulcerated surfaces which were for the most part covered with adherent fibrino-purulent pseudomembrane. This condition was especially well marked over the buttocks and in the sacral region. On attempting to turn the body on the side, a thin watery fluid containing flakes of mucous, ran from the mouth. The peripheral lymph glands were not noticeably enlarged. The teeth and gums were in a frightful state. The teeth, most of them decayed, were loose and hung loosely by their blackened stumps in pigmented suppurating gums. The subcutaneous fat was well developed. Internally (mesially) to the wound in the thorax was a small subcutaneous abscess containing a greenish-yellow caseous pus. It measured about 2 cm. in diameter.

The lungs collapsed incompletely when the chest was opened. In the left side there were old anterolateral, posterior, and apical adhesions, and but a small amount of pleural fluid containing some blood. On the right side were numerous lateral, posterior, and apical adhesions and also a considerably increased pleural exudate. The lobes of the lungs were bound together by old adhesions. The surfaces of all the lobes of both lungs had a distinctly shotty feel which was due to the presence of innumerable miliary tubercles which were present, as section showed, everywhere throughout the pulmonary tissue. The lungs were quite edematous and moderately congested. Congestion was more evident in the left lung. At both apices were old apical scars. In the mediastinal fat and areolar tissue there was a considerable amount of black pigment which ran in lines down upon the upper surface of the diaphragm, where at one or two places it formed irregular patches. There was no evidence of thymus tissue. There was no increase of pericardial fluid. In the right heart was a mass of agonal (?) clot of considerable size. The valves were apparently normal except the aortic, upon the cusps of which were a few fine fibrinous vegetations. The aorta showed nothing else than a moderate degree of fatty degeneration with occasional areas of early atheroma. The spleen was large, soft and friable. The capsule was smooth and the edges rounded. The Malpighian corpuscles were just visible on the cut surface. The left kidney (225) was enlarged and pale. Its surface was irregularly, though only moderately, lobulated. The capsule was adherent. The surface was generally gray, mottled with lines of congested capillaries. The whole organ was moderately edematous. The glomeruli were not visible. The line of demarkation between cortex and medulla was not distinct. In the medulla at two points were small grayish nodules, which resembled miliary tubercles. The general consistency of the organ was increased.

The left adrenal was congested and showed evidence of increased lipid change. A cortical adenoma was present in the medullary portion. The medulla was possibly hypertrophied.

The right kidney (110) was small and also irregularly lobulated. The middle third of the anterior half of the organ had all the appearances (externally) of the left kidney, but the rest of the organ was much paler and to the touch seemed to contain large cysts. On section it was discovered that practically the whole organ was the seat of a chronic suppurative process which had given rise to spaces filled with a cheesy, yellowish material. The pelvis, ureter and bladder showed no changes. The prostate was unusually friable and partly cystic, possibly tuberculous, though there was no good evidence of it. The seminal vesicles were apparently healthy. In the right epididymus was a focus of caseation. The testicles were normal. The right adrenal was small and cavitated. The liver was of about normal size (1,750 grms.). It was gray with a yellowish tinge, and marked with indistinct lines of congestion. There were the small tags of old adhesions upon its surface, especially on the right. Scattered here and there under the capsule were small rounded areas which resembled small tubercles. There were others of these within the substance of the organ. The cut surface was moist, gray and irregularly mottled. There was a large angioma in the right lobe (1×1.5 cm.). The bile ducts were patent, and there were no gall stones. There were old adhesions between the gall bladder and the gastro-colic omentum. The lymph glands in the celiac region were completely calcified and formed irregular masses. Scattered here and there in the mesentery, as a rule about halfway between the gut and the origin of that membrane, were calcareous masses irregular in outline, between which and the origin of the mesentery there were lines of hyaline fibrosis. The omentum was adherent to the parietal peritoneum at the

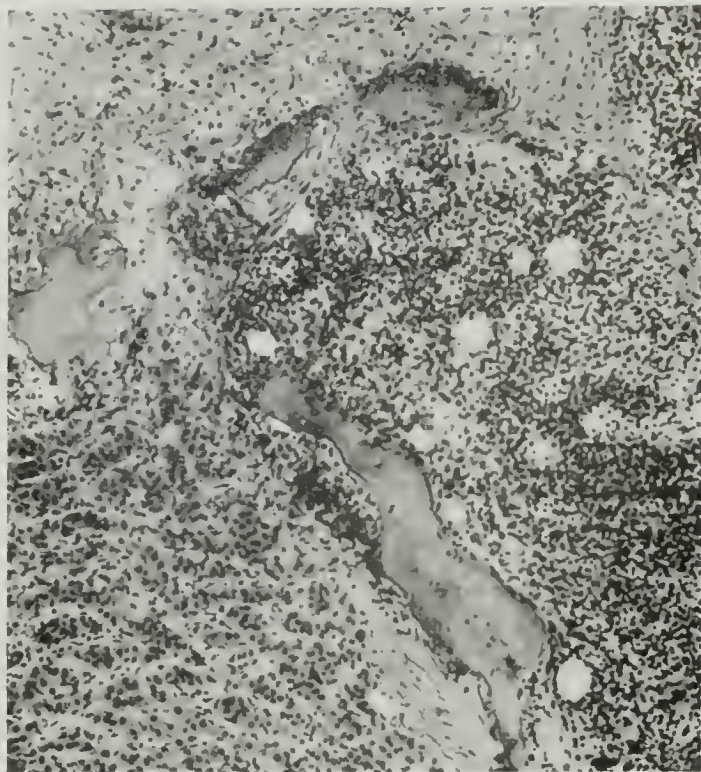


Fig. 1.—A section showing the presence of true bone and bone-marrow in the adrenal.



Fig. 2.—A section showing massing of small round cells about the bone in the adrenal.

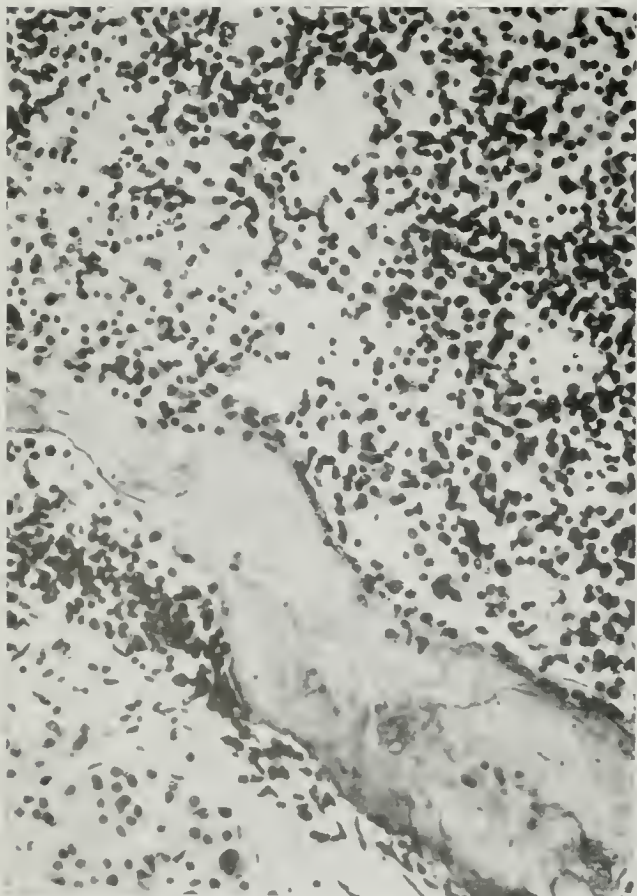


Fig. 3.—A section illustrating the same things as Figs. 1 and 2, but with greater magnification.

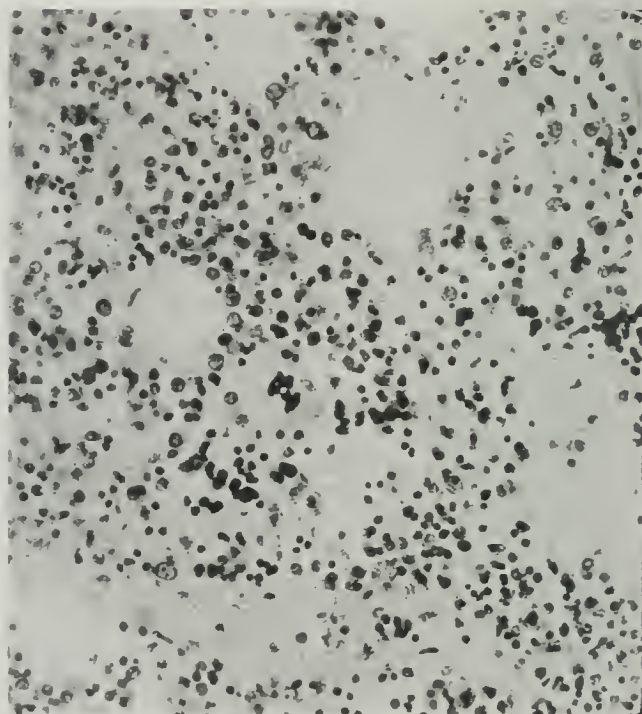


Fig. 4.—A section of the marrow in the adrenal, showing blood sinuses, and one megakaryocyte.

right internal ring and at the right side of the bladder. The appendix was imbedded in a post-cecal pouch and folded sharply upon itself so that the tip and the origin were close together at the mouth of the pouch. The tracheal and bronchial lymph glands were enlarged, deeply pigmented and edematous. The thymus was absent. The trachea showed nothing abnormal. The intestine showed nothing remarkable. The brain (1,235 grms.) showed a marked diffuse edema, which was most evident over the vertex. In many of the sulci the fluid was clouded and in some places almost purulent. There was no distinct evidence of tuberculosis though there were occasional scattered areas which were suggestive. The ventricular fluid was increased. The brain was remarkably so. The cord also was very soft and almost diffuent. The pituitary was apparently normal. The pineal was edematous and had a calcified center.

The anatomic diagnosis was, generalized miliary tuberculosis; Tuberculous costochondritis; Acute vegetative aortic endocarditis; Tuberculous pyonephrosis;

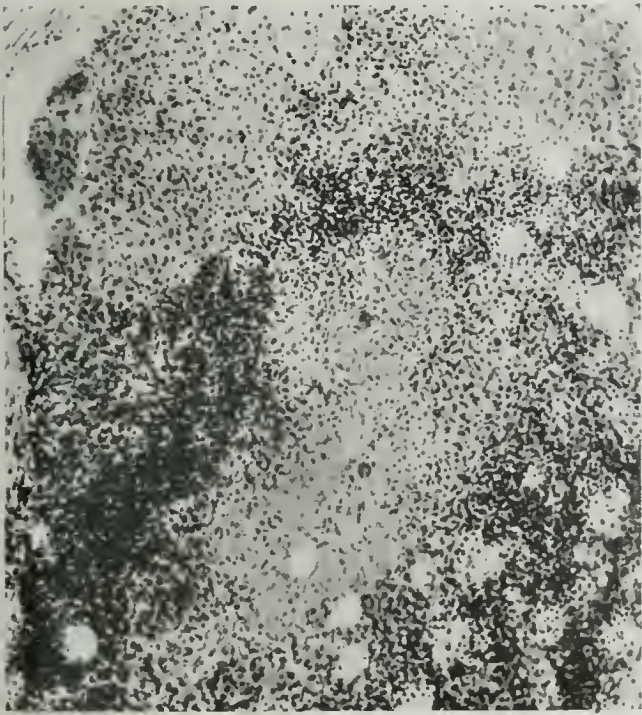


Fig. 5.—A tuberculous area in the marrow in the adrenal.

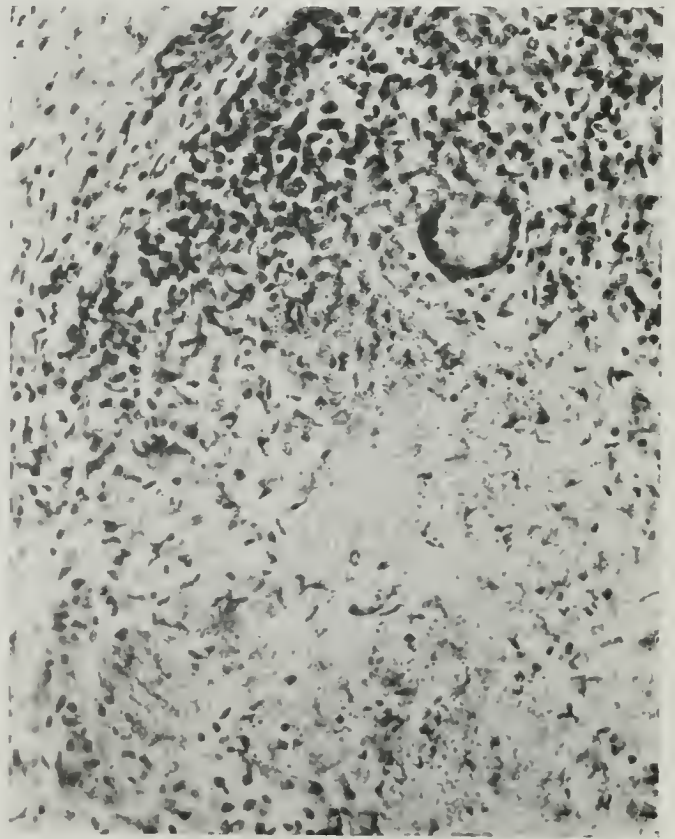


Fig. 6.—A tuberculous giant cell in an area in the adrenal.

Serous meningitis; Pyorrhea alveolaris; Varicose leg ulcer; Mediastinal anthracosis; Calcified mesenteric lymph glands; Adrenal tumor (?).

The histologic examination of the tissues preserved at postmortem confirmed the anatomic diagnosis, except in the case of the left adrenal in which region a most interesting condition was observed, and one which, so far as I have been able to discover, has been mentioned but a few times in medical literature.

The adrenal had been fixed in Zenker's fluid, washed, cut in thin slices, embedded in celloidin, cut and stained with hematoxylin and eosin (Delafield's) and in Mayer's acid hemalaun. In cutting the sections, the technician had realized that the tissue contained a small amount of something which grated upon the knife.

Upon examination nothing remarkable was encountered in the cortex. Aside from a moderate lipoid change, and the presence of a few foci of so-called ade-

nomas, there was nothing worthy of note, except at one or two places where a few spicules of bone were observed surrounding a cellular structure which resembled bone marrow. The medulla was evidently hyperplastic and at one point was the area which at postmortem had been described as possibly an adenoma. This turned out to be a mass of myeloid tissue almost completely surrounded by well formed bone, and containing within it at more than one point several miliary tubercles. Further search in other sections brought to light occasional tubercles in the cortex of the adrenal, usually closely associated with the myeloid areas. In the cortex these structures measured 1×0.5 mm.; in the medulla, the one large mass of myeloid tissue measured 6×3 mm.

Careful study of these anomalous foci gave definite evidence that they were actually and typically bone marrow surrounded by bone, as the figures will show. There was a rich capillary network with endothelial lining between which were numbers of lymphocytes, leucocytes, myelocytes, and giant cells, beside developmental forms of red blood cells. The bone was lined with periosteum and endosteum.

The fact that the combination of tuberculosis of the adrenal associated with bone and myeloid tissue (tuberculous osteomyelitis of the adrenal) is rare, is less interesting than the reason why the bone appeared. I am of the belief that the fundamental lesion was a chronic tuberculous one, and that the bone and medulla formation was secondary to the irritation produced by the tubercle bacilli. It seems to me that the reaction of the tissue to the tuberculous irritation showed itself in hyperplasia of the supporting tissue with only slowly progressing necrosis and slight deposition of lime salts, a combination of circumstances which led to the proper conditions for functional change on the part of the connective tissue cells.

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A CARDIAC FORMULA FOR THE RELATIVE MEASUREMENTS OF THE VALVULAR ORIFICES*

BY ROBERT A. KEILTY, M.D., PHILADELPHIA, PA.

THE purpose of this presentation is to offer a working basis for the determination of cardiac valvular stenosis or insufficiency. In an autopsy technic the incisions for opening the heart vary to a wide extent, each individual operator having a method of choice. I am in the habit of opening the left ventricle first, exposing the aortic and mitral in order, then the right ventricle, exposing the tricusped and pulmonary orifices. Thus aortic, mitral, tricusped and pulmonary may be examined in order. Hereafter in this paper these openings are designated A. M. T. P.

The most accurate method of determining valvular stenosis or insufficiency is by means of measuring the various valve orifices after they have been incised. The normal diameters as given by different authors vary to some extent. For example, in centimeters, A 8.0, M 10.5, T 12.0, P 9.0, A. J. Smith; A 7.7, M. 10.4, T 12.0, P 8.9 and A 8.0, M 10.9, T 12.7, P 9.2, Mallory and Wright after Krause; female A 7.3, M 9.7, T 12.9, P 8.5, and male A 8.2, M 11.1, T 13.7, P 9.1, Shennon.

These measurements were always confusing to me until a few years ago when I noticed the following ratio was almost a constant factor, A 7, M 9, T 11, P 7, 7-9-11-7. These are the only figures one needs to remember. In the same proportion the orifices vary up to A 9, M 11, T 13, P 9, 9-11-13-9. Thus a heart 8.5-10.5-12.5-8.5 for example would be within normal proportions. In working such a formula the weight of the heart must be taken into consideration. In a small heart of 300 grms. we might expect to find 7-9-11-7, while in a large heart of 600 grms. 9-11-13-9. There may be said to be a variation within the normal, as applied to stenosis or insufficiency, of the following 7-9, 9-11, 11-13, 7-9.

It will be found that the measurements of the aortic and pulmonary valves are about the same, the pulmonary always a little larger. The mitral is about 2 cm. wider than the aortic and the tricusped about 2 cm. wider than the mitral.

In aortic stenosis, for example, with a large heart we might expect a valvular formula similar to this A 6, M 11, T 13, P 9, 6-11-13-9; in aortic insufficiency with a heart of the same weight A 11, M 11, T 13, P 9, 11-11-13-9.

In view of the great number of hearts, at least as found at autopsy, which show right-sided dilatation to some degree the measurement of the tricusped will vary more than of the other orifices. This variation in most instances will be found relative rather than organic and may be expressed in figures 7-9-14-7.

The figures which I have given are not exact, that is, they are not the result of the measurements of a thousand hearts but they are offered as a work-

*From the McManes Laboratory of Pathology of the University of Pennsylvania. Presented before the Pathological Society of Philadelphia, Jan. 27, 1916.

ing basis, easily remembered, for the determination either of stenosis or insufficiency at the time of an autopsy.

CONCLUSIONS.

1. In the following order A-M-T-P a cardiac valvular formula in centimeters 7-9-11-7 may be used as a practical working basis for the determination of stenosis or insufficiency.
2. This varies in accordance with the size of the heart within these limits 7-9, 9-11, 11-13, 7-9, and rarely goes above or below.
3. The variations affect all valves in proportion.
4. Upon finding any marked deviation in centimeters in one or two valves from the formula as given one may be justified in concluding either stenosis or insufficiency is present in a given case.

THE COMPRESSION SYNDROME OF THE CEREBROSPINAL FLUID

BY G. W. BROCK, ATLANTA, ILL.

IN 1903 Froin reported three cases in which on withdrawal of the cerebrospinal fluid, the fluid was yellow in color, coagulated spontaneously, and contained numerous cellular elements, mostly leucocytes.

Since that time there have been some thirty-five cases or more reported in the literature.

The significance of this syndrome was first brought to my attention by Dr. Charles Louis Mix, and in the "Murphy Clinics" of April, 1915, he gives a summary of the cases reported up to that time, including one of Dr. Murphy's cases.

In the *Journal of the American Medical Association* for July 10, 1915, is an extract from the *Wiener klinische Wochenschrift*, of two tumor cases showing this syndrome reported by Schlesinger, one due to an extradural tumor, the other an angiosarcoma of the cauda equina.

The conditions leading to this syndrome may be any traumatic or pathologic process which cuts off the lower portion of the spinal canal, either completely or partially, forming a separate sac, the obstruction to the lumen of the canal usually being somewhere between the sixth thoracic and third lumbar vertebræ.

These conditions include: Trauma with hemorrhage in which the sleeve-like clot becomes organized; inflammatory reactions, with the formation of a fibrous tissue barrier, the inflammation being in the form of an acute meningitis, a pachymeningitis, or meningomyelitis, due to irritants injected from without, as stovaine in one case; or commonly bacterial in origin, including tuberculosis and syphilis.

Tumors or cysts of the conus terminalis or cauda equina or a Pott's disease with pressure, may be the etiologic factor.

Any one of the above lesions when occurring in the area of the lower segments of the cord or the cauda and their coverings, may produce this syndrome of xanthochromia, spontaneous coagulation, and hematology.

The coagulation may be immediate, causing stoppage of the needle, or within a period of two or three hours, due to the amount of fibrin present in the exudate.

The cellular elements are most commonly entirely made up of lymphocytes.

Subsequent punctures may give a spinal fluid that varies in color from clear to a marked yellow; coagulation may be absent, when at some other puncture the fluid coagulates in the lumen of the needle; and the number of cells may vary from none to a numerous lymphocytosis.

This variance in the findings in the examination of the cerebrospinal fluid is due to the amount of walling-off in the inflammatory conditions, or the so-called "ball-valve" action, mentioned by Mix, as exerted by some tumors, which may permit the cerebrospinal fluid to pass the obstructing lesion, or make of the distal portion of the spinal canal a closed sac.

Sicard and Descomps were the first to recognize this meningeal pouch to be the essential physical factor in producing this change in the cerebrospinal fluid.

Three types of cases have been described: First, Acute cases of meningitis, some presenting the syndrome of a Landry's paralysis; Second, Paraplegia either spastic or flaccid, depending on the level affected, most of the cases being due to a pachymeningomyelitis of the conus terminalis. The symptoms, besides those of paralysis, are variable, disturbance of sensation and sphincteric control, with severe pain being most constant; Third, Tumors and cysts, either of the conus terminalis, cauda equina or the dura.

The duration of the symptoms leading to the production of this syndrome are reported as from a few days to seventeen years, although in long-standing cases it would be impossible to say how long this syndrome had been present.

Klienberger has reported three cases, two due to tumor, and the third as probably due to dementia paralytica, the etiology of the following case:

CASE REPORT.—M. L. P., male, age 34. Admitted to the Peoria State Hospital, Oct. 17, 1913, and assigned to the service of Dr. J. K. Pollock, physician in charge of the male receiving service.

Occupation, farmer; civil condition, married. The attack was of one year duration. No history of alcohol.

Physical Examination.—Well built, weight 160 pounds; pressure sore on right scapula and right knee, marked tremor, loss of sphincteric control. Gait very unsteady, with feet far apart. Pupils unequal and irregular, but reacted to light and accommodation. Deep reflexes exaggerated, the left knee more so than the right; Gordon and Babinski absent.

Mental Examination.—Disoriented for time and place, judgment extremely deficient, some grandiose ideas concerning wealth. Diagnosis, Dementia paralytica of the dementing type.

Three days after admission a lumbar puncture was made, which gave the following findings: Amount of cerebrospinal fluid withdrawn 15 c.c.; color lemon-yellow; Noguchi and Nonne-Apelt tests both positive; Lange's gold solu-

tion reduced to a white color in dilutions of 1-20, 1-40, and 1-80; cell count 50 cells per cm.; lymphocytes.

After about one hour the fluid coagulated in the test-tube into an almost solid mass. Wassermann strongly positive. Autopsy denied.

THE PRESENT STATUS OF THE ADRENAL PROBLEM*

BY R. G. HOSKINS, PH.D., CHICAGO, ILL.

TO attempt to formulate a conception of the significance of the adrenal glands in the existing state of the literature is a somewhat precarious undertaking. This literature although extensive is by no means consistent nor complete. One can find recorded data that support almost any possible conclusion. His final conception rests therefore, to a considerable extent, on his selection and evaluation of more or less conflicting evidence.

From the confused maze, however, a few facts of major importance stand out unquestioned. Before taking up the present status of the adrenal problem to afford a background these may be briefly reviewed. Although the adrenals had long been recognized as definite anatomical structures, the first significant contribution to our knowledge of their function was made by Addison, an English clinician. In 1855 he reported that adrenal deficiency gives rise to a characteristic syndrome the cardinal features of which are muscular weakness, circulatory depression, gastrointestinal disturbances and deposition of a dark pigment in the skin and mucous membranes.¹ This discovery constitutes the most important evidence as to adrenal functioning even as we know it today. It also raises the most insistent question that still remains to be answered, namely,—What is the specific explanation of this complex; Why does an animal deprived of its suprarenal tissue inevitably die?

The next significant discovery was made by Oliver, another clinician. In an investigation of the effects of various gland extracts on his patients he discovered that suprarenal material is especially potent. In collaboration with the Edinburgh physiologist, Schaefer, he then undertook a systematic investigation of the properties of suprarenal extracts. It was discovered among other things that such extracts have a remarkable stimulating effect upon the circulatory apparatus, causing great augmentation of blood pressure. These observations were published in 1894-5.² The discovery was made independently by Szymonowicz, however, and published in 1896.³

The isolation of the so-called "active principle"—better *an* active principle—of the extract was soon after accomplished. Abel in 1897 secured it as a benzoyl addition product which he called "epinephrin."⁴ Takamine and Aldrich in 1901 succeeded in isolating it as a pure crystalline substance.⁵ They called it "adrenalin" which term was adopted as a trade name by the

*From the Laboratory of Physiology of the Northwestern University Medical School.
Read before The Institute of Medicine of Chicago, January 28, 1916.

pharmaceutical firm by which they were employed. As a matter of usage adrenin seems now to be the favorite term for the substance. It belongs chemically among the benzene ring compounds, as is indicated by its name dioxyphenyl-ethanol-methyl-amine. Its composition suggests that it may be derived from tyrosin, one of the amino acids obtained in protein digestion. It is formed in the medulla but not in the cortex of the suprarenal.

The next outstanding discovery was made by a group of English investigators,—particularly Elliott.⁶ His elaborate paper of 1905 has become a classic. He showed conclusively that adrenal extract stimulates selectively the neuro-cellular endings of the sympathetic nervous system wherever these are found. The effect—at least of physiologic quantities—depends in any organ particularly upon three factors: If the organ receives no sympathetic fibers no effect is produced. If the sympathetic fibers are relatively little used in every day life the effect of adrenin is correspondingly restricted. The effect is either augmentation or depression of the function of a given tissue depending upon whether its sympathetic fibers are excitatory or inhibitory. Thus, the uterus is stimulated whereas the intestine is depressed. This discovery seemed to many largely to settle the adrenal problem. But to this point we shall revert.

Another recent discovery that will probably be recognized as of major importance is that the adrenals are particularly influenced by emergency conditions. A conception of the “emergency function” of the glands based largely upon work done in his laboratory has recently been formulated by Cannon.⁷ Various aspects of this function have been worked out and published in a series of joint papers since 1911. Cannon in studying the activities of the alimentary tract had noticed that an outburst of emotion, either fear or anger, resulted in a checking of peristalsis. This might well be due to an outflow of impulses throughout the sympathetic nervous system,—a phenomenon that is well known to occur under such circumstances. However, the investigator was struck by the fact that the depression of peristalsis persisted for some time after all signs of emotion had subsided. Adrenin was known to inhibit peristalsis. The adrenal glands were known to receive sympathetic fibers and hence supposedly to receive a share of the sympathetic impulses. It was suspected then that the prolonged alimentary depression might be due to an after discharge of adrenin from the excited glands. Direct research confirmed the suspicion.

Cannon in collaboration with de la Paz first studied the effect of violent emotions upon the adrenin content of the blood.⁸ The method used was briefly this: A cat was bound to a comfortable holder. Then under local anesthesia a femoral vein was exposed and opened. This permitted the introduction of a long flexible catheter into the vena cava so as to secure a sample of blood from the level of the adrenal veins. The animal then had its sensibilities harrowed by the threatening presence of a barking dog. When the angry passions of the cat were sufficiently aroused a second sample of blood was secured. The two samples were immediately compared as to adrenin content. To find a suitable test object that could be used with defibrinated blood was for some time a baffling problem. Finally, however,

strips of longitudinal muscle of the cat's intestine were found to be suitable. These immersed in normal blood will undergo spontaneous, long continued, rhythmic contractions. If a trace of adrenin is added, however, the contractions immediately cease. By means of this test the two samples of blood were compared. That obtained while the animal was quiet showed no detectable adrenin. That, however, from the excited animal caused a marked depression of the intestinal strip. By suitable means it was shown that the depressing substance actually was derived from the adrenal glands. It was concluded, therefore, that during times of emotional stress the adrenal glands are stimulated to pour into the blood stream enough adrenin to exert a significant influence.

The problem then presented itself: Is this adrenin discharge purely a psychic phenomenon? In other words, could it be produced in an unconscious subject? That problem was investigated by Cannon and Hoskins.⁹ Since pain is the most common source of emotion its effect on the adrenals was investigated. It was not advisable to subject the experimental animals to actual pain, however, hence its physiologic equivalent, sensory stimulation under anesthesia was employed. A stimulating electrode was adjusted to the sciatic nerve. Then a sample of blood was secured by the method previously described. A strong tetanizing current was applied to the nerve and after a short period of stimulation a second sample of blood secured. In the meanwhile it had been found that segments of rabbit intestine are more sensitive and more easily secured than are intestinal strips.¹⁰ In this and subsequent researches along this line, therefore, such segments were used for the tests. It was found just as in the earlier experiments that blood secured after stimulation of the sciatic gave a clean-cut adrenin reaction. These results have been confirmed by other investigators, notably Elliott,¹¹ who has observed that sensory irritation results in exhausting the adrenin content of the gland. This fact has an obvious bearing on clinical practice. Any severe surgical operation is essentially a repetition of the experiment described. Adrenal exhaustion, therefore, is one of the immediate results of any such operation. Similarly, it was found that asphyxia causes adrenal discharge,—a fact that has also been confirmed in various ways.

Since this adrenal discharge may be purely reflex, and since reflexes are usually of direct benefit to the individual, the question arises: Of what use is the arrangement? McDougal has suggested that emotions are the mental representation of bodily activities: anger represents combat, fear represents flight. In case combat results, pain is experienced. Either flight or combat causes partial asphyxia, that is, breathlessness—at least until the body has had time to adjust to the conditions. All of these cause adrenal discharge. The adrenal discharge might be supposed, therefore, to aid in some way in strenuous muscular activity; that is, to integrate the body for muscular response to the stressful condition which caused the discharge.

As a matter of fact, the injection of adrenin does have a number of effects that aid in violent muscular activity. A quiescence of the whole digestive system results and the blood is shifted from the splanchnic circulation to the active muscles, and to the nervous and respiratory organs. The cir-

culatation in these organs is further increased by a rise of blood pressure resulting from vasoconstriction of the splanchnic area and often of the skin, as well as by augmented cardiac discharge. In a direct investigation Hartman¹² working in Cannon's laboratory has recently shown that a given dose of adrenin simultaneously dilates the vessels of the muscles and constricts those of the splanchnic organs. A discharge of dextrose occurs, leading to hyperglycemia, whereby the laboring muscles obtain a better supply of energy material. This hyperglycemia has been observed as a direct result of strong emotion as well as of artificially augmented adrenin. The well-known bronchodilator effect of adrenin is of obvious utility in aiding free breathing whereby the oxygen necessary to violent activity is secured. Most striking of all, however, the laboring muscle itself is directly benefited. Cannon and Nice¹³ observed that after the injection of adrenin or stimulation of the splanchnic nerves to cause adrenal discharge, the efficiency of a fatigued muscle is improved, sometimes 100 per cent. Gruber¹⁴ has found that recovery from fatigue is greatly hastened by the injection of adrenin. The explanation of this fact is not definitely known, but a hint is offered by Evans and Ogawa's observation¹⁵ that in the heart at least the assimilation of oxygen is increased by this substance. Hartman's observation that adrenin has a selective vasodilator influence in muscle also comes to mind. Cannon, Gray and Mendenhall¹⁶ have noted another phenomenon which is of further use to the individual in case the stressful condition results in bodily injury: Adrenin lessens the coagulation time of the blood. This is true, however, only within physiologic limits,—for such quantities as can be obtained by stimulating the splanchnic nerves. In case greater quantities are employed, the coagulation is interfered with. With proper regard to this fact, adrenin might well come into frequent clinical use in combating hemorrhage. Cannon suggests that the pains of labor are of direct use to the mother in causing adrenal discharge and thereby protecting her from post-partum hemorrhage. One thinks also of the stimulating effect of adrenin on the uterus. On the whole it seems to be established that in times of stress the adrenal glands are stimulated to discharge a secretion that is of use in integrating the body to cope with emergencies.

The question may now be raised: What part do the adrenals play during periods of more placid existence? A few years ago a very satisfactory theory could be constructed about as follows: An animal deprived of its adrenal glands dies. The blood pressure falls indicating that the sympathetic nervous system has failed in its function. Injection of adrenal extract causes a marked rise of pressure; therefore, the function of the adrenal glands is to maintain the tonus of the sympathetic nervous system. This might be designated the "tonus theory." Although several facts are in contradiction to it the theory still has considerable currency.¹⁷

The first question that arises when one considers the theory is this: Is there present in the blood stream as it leaves the heart enough adrenin to exert any appreciable influence on the sympathetic nervous system? In the earlier literature occur various statements which indicate that the blood does contain considerable quantities. The concentration is given as, for example,

one part in 10,000,000. But it is interesting to note that as the technic of such determinations has improved the dilution of adrenin has continuously approached infinity. The best method now known for such tests is to run the suspected blood through the capillary bed of a frog's legs and note the rate of outflow. Trendelenburg, the recognized authority on this method, has recently reported that the concentration of adrenin in arterial blood is at most not more than one part in one or two billions or only one-fifth enough to exert any appreciable effect on the sympathetic system of a mammal.¹⁸

The tonus theory can be considered from another point of view. It assumes that the adrenals are constantly supplying enough secretion to stimulate the pressor mechanisms. This being true the addition of a little more of the drug by vein should augment this pressor influence and send the tension higher. As a matter of fact quite the opposite occurs. Such injections characteristically lower the pressure. This observation was made by earlier investigators, notably by Moore and Purinton,¹⁹ but was ascribed to impurities in the material used. In 1912 Hoskins and McClure²⁰ reinvestigated the matter using a supposedly pure drug. Their experiments were made on dogs. The depressor effect was easily demonstrated. The fact was confirmed for cats by Cannon and Lyman.²¹ In 1913 Hoskins and McPeck²² showed that light massage of the suprarenals leads to a similar fall of pressure.

Again,—if the adrenals are pouring into the blood stream enough secretion to hold up pressure sudden occlusion of the adrenal veins should cause a prompt fall of pressure. As a matter of fact such procedure leaves the pressure exactly where it was before.²³ It is a matter of hours before a fall occurs whereas circulating adrenin is destroyed within a minute or two.

Hoskins and McClure²⁴ have tested the theory in another way. If adrenin be injected into a vein at very small but gradually increasing rates at first no effect at all is to be seen. Then various changes occur. One of the earliest is depression of intestinal peristalsis. If simultaneous tracings of peristalsis and blood pressure are taken it is found that the gut is paralyzed before any rise of blood pressure occurs. One need not point out the futility of an arrangement that could maintain blood pressure only at the expense of gastrointestinal paralysis. Other evidence of similar tenor could be offered but this may suffice. Attractive as the tonus theory was it is no longer tenable.

But the fact remains,—adrenal extirpation is fatal and the final symptoms include a failure of functions that are under sympathetic control. Elliott²⁵ has offered the interesting suggestion that a minute quantity of circulating adrenin is necessary, not to stimulate the sympathetic system but to maintain its irritability. That is, the terminal neurocellular substance of the sympathetic system in the absence of adrenin is no longer able to transmit impulses. This possibility also was investigated in our laboratory. It seemed to us that animals at the point of death, such as Elliott worked on, are not capable of giving any very significant information. All sorts of secondary factors may enter into the experiment. If sympathetic failure is a characteristic feature of the syndrome, it should appear at an early stage. It was found that at a time when the animal deprived of its adrenals is showing marked evidence of that fact,—when it can scarcely sustain its own weight,—its vasomotor system responds to stimulation

perfectly well.²⁶ Vasomotor—i.e., sympathetic failure, therefore, is to be regarded as a secondary feature. Both muscular and cardiac weakness precede it.

It would seem probable that if a trace of adrenin is essential for sympathetic functioning, the vasomotor reactions should be improved if an animal previously deprived of its adrenals were to receive a continuous injection of very dilute adrenin for, say half an hour. In carrying out this experiment the surprising fact was noted that such injections often actually impede sympathetic functioning. In some cases pronounced block was demonstrated.²⁷ This observation, which was confirmed many times both in normal animals and those deprived of their adrenals, seems to dispose of the alternative theory that adrenin facilitates sympathetic functioning. The conclusion follows then that the remarkable effect of larger doses of adrenin on the sympathetic system is of use in emergencies only.

The possibility still remains that minute quantities of adrenin are necessary for the metabolism of other tissues, for example, as Crile supposes, the brain cells.²⁸ If such were the case,—if adrenin failure were the significant feature in adrenal deficiency—it should be possible by continuous injections of adrenin to preserve the life of the operated animal. As a matter of fact no significant prolongation of life can thus be achieved.²⁹ Laying all theories aside and facing this fact, one can scarcely escape the conclusion that adrenin has no essential connection with the Addison syndrome or its laboratory equivalent,—that it is merely a reserve resource for use in emergencies.

The cause of death then in adrenal deficiency remains still an unsolved riddle. We are led back to the theories that prevailed before the potency of adrenal extracts was discovered. Do these glands perchance have a detoxicating function? Is the essential feature in adrenal death a toxemia? If so, the toxin should be detectable,—particularly since it is often potent enough to kill a dog within a few hours. There are on record some early observations which indicate that such a toxin can be demonstrated. Abelous and Langlois³⁰ reported that the blood of guinea-pigs dying after adrenal extirpation is fatal to frogs. In view of the possibility of the formation of various decomposition products, however, the burden of proof is on the experimenter to show that the toxin is specifically due to adrenal failure. Negative results in such experiments are more significant than positive. The matter has recently been under investigation in our laboratory. The work is not entirely finished but enough has been done to indicate that the blood of dogs that have just died of adrenal deficiency is in no degree toxic to frogs. Other observers have failed to detect a toxin by transfusion experiments.

We are finally thrown back then to the vague conception of a failure of tissue metabolism as the most probable cause of death. The tissues that first exhibit weakness are the cardiac and muscular. The most satisfactory theory for the time being, therefore, would seem to be that the adrenals contribute to the blood stream some unknown substance necessary to the metabolic processes of these active tissues. The best evidence indicates that this hypothetical substance is derived, not from the adrenal medulla which supplies adrenin, but from the cortex of the gland. At any rate in animals in which the two parts of the gland

are separate, death follows when the cortex homologue is removed leaving the chromaffin tissue unharmed.³¹

One other line of evidence bears on the question as to the function of the cortex. In various sorts of sex-gland anomalies hypertrophy of this tissue is observed. Most striking, perhaps, is the fact that sexual precocity in children has been shown to be accompanied by marked adrenal hypertrophy or hypernephromata.³² In some experiments recently made³³ evidence has been secured that feeding suprarenal substance to young animals leads to hypertrophy of the testes. Further experiments along this line may lead to significant results.

In conclusion it may be said that the fundamental question remains yet to be answered: Why does the removal of the adrenal glands cause death? The trend of the evidence now available suggests that muscular metabolism is at fault. If that be true the solution, like that of many other of the most puzzling medical problems, rests in the hands of the biological chemists.

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LABORATORY METHODS

The Results of Quantitative Albumin Determinations from 6000 Cases of Suspected Tuberculosis*

BY M. L. HOLM, M.D., AND E. R. CHAMBERS, B.S., LANSING, MICH.

DURING the last five years considerable attention has been given to the determination of albumin in sputum by both qualitative and quantitative methods and numerous contributions to the literature on this subject have been made by several writers. A review of this literature is somewhat interesting but to one without extensive personal experience such review would seem rather confusing. The summary of conclusions drawn by different writers varies as to the value of albumin findings from an opinion that the determination has no practical value in the study of respiratory diseases to an opinion that it is one of our most valuable aids in the diagnosis of respiratory affections. There appear to be just reasons for these varying conclusions based upon several factors. Some of the publications are the result of study from a number of cases entirely too small to justify any conclusion whatsoever. Some writers appear to have been biased in their opinion either before their work was begun or by their early results and have thus failed to give logical consideration to their findings as a whole. Most important, however, is probably the general lack of uniformity in methods pursued and the personal equation influencing the delicacy of the reactions or determinations to be noted. Thus one writer reports albumin practically always present in sputum, regardless of the disease and another found albumin distinctly present in only a little over 60 per cent of cases of open tuberculosis, doubtful in 27 per cent, and entirely absent in 9.4 per cent of tuberculosis patients.

It must be conceded that almost all sputa and even normal saliva contains albumin-like bodies that can be detected or actually measured by means of certain well-known chemical processes. It is likewise true that after careful separation of mucin many sputa, even though containing distinct quantities of albumin, may fail to give certain of the albumin reactions especially to the less experienced observers.

Some time ago we† published a method for the quantitative determination of albumin in sputum with potassium ferrocyanid which is essentially as follows: A quantity of sputum, not less than 10 c.c., is collected in a dry receptacle in the usual way. (Samples containing blood should be rejected, as blood invariably contains albumin.) After smears for microscopic examination have

*From the Lansing (Mich.) Clinical Laboratory and the Upper Peninsula Hygienic Laboratory.

†*Jour. Am. Med. Assn.*, lxii, 20.

Jour. Michigan Med. Soc., April, 1914.

been made a quantity of sputum not exceeding 10 c.c. is poured into a 50 c.c. graduated glass-stoppered cylinder. To this is added three times its volume of water containing 1 per cent acetic acid, diluting the sputum to 25 per cent. After vigorous shaking with the stopper in place, the mixture is filtered through filter paper directly into a graduated centrifuge tube and 10 c.c. collected. To this is added 5 c.c. of 5 per cent solution of potassium ferrocyanid in water and the tube whirled in a centrifuge for five minutes at average speed. The amount of albumin is most conveniently recorded in volume per cent, each 0.1 c.c. on the tube being 4 per cent by volume after correcting for the original dilution. Absolute accuracy is impracticable in this work, so only approximate results should be recorded. If desired, the volume per cent may be calculated to weight per cent according to the method of Purdy for urinalysis.

We have since the first publication employed this procedure on over 6,000 sputa and it has proved entirely satisfactory. Various modifications that have been proposed by others have not been found satisfactory by us.

The 6,000 cases offered here are taken in consecutive order from sputa received from general practitioners in Michigan and represent what the average physician submits from what he considers suspected cases of tuberculosis. Special examination from selected cases are not included in the table. For the purpose of convenience we have divided these cases into two groups, namely, those containing tubercle bacilli and those not containing tubercle bacilli. Out of the total of 6,000 sputa examined, tubercle bacilli were present in 1,412 and absent in 4,588. The albumin determinations gave results as follows:

PRESENT		TUBERCLE BACILLI		ABSENT	
1,412	Sputa Examined	4,588	
3%	Albumin Absent	40.0%	
97%	Albumin Present	60.0%	
Quantitative Determination					
5.0%	Below ½% by volume	14.4%	
3.0%	Between ½-2% by volume	4.0%	
7.0%	“ 2-5% “ “	8.0%	
10.0%	“ 5-10% “ “	9.2%	
36.0%	“ 10-25% “ “	15.4%	
26.0%	“ 25-50% “ “	7.8%	
10.0%	Above 50% “ “	1.2%	

In addition we have examined discharges from a considerable number of abscesses as well as secretions and exudates from various other parts of the body. Such abscesses have generally shown albumin ranging in volume from 10 per cent to 35 per cent. Two cases of pulmonary edema gave albumin over 50 per cent by volume. Twenty-eight cases of pneumonia in various stages gave albumin ranging from 10 per cent to 50 per cent by volume. Eleven cases of pleuritic effusion, diagnosed as tuberculosis gave albumin above 50 per cent by volume.

One case of tuberculosis of the bowel gave about 1 per cent volume of albumin in the stool and seven cases of tuberculosis of the kidney showed from 10 to 25 per cent of albumin by volume in the urine.

Our experience indicates that normal saliva, urine, feces and stomach con-

tents, following an Ewald test meal, do not show albumin by the method we have employed.

In urine a negative albumin reaction appears to us to preclude active tuberculosis of the kidney and we believe albumin in the urine is constantly associated with active tuberculosis in any part of the urinary tract. The same is probably true of the bowel. Several writers state that the finding of tubercle bacilli in the stool is conclusive evidence of intestinal tuberculosis. This appears to us to be an error as we have been able to show that tubercle bacilli can be demonstrated, with sufficient search, in the stool of practically every case of open pulmonary tuberculosis. Some tubercle bacilli are inevitably swallowed and pass through the alimentary tract. Soluble albumins swallowed do not normally appear in the stool and the appearance of such albumin in the stool generally indicates that an albuminous exudate is entering the lumen of the intestine lower down.

In dealing with sputa the interpretation of results is somewhat difficult. The composition of what is ordinarily termed a sputa may be rather complex, including saliva, discharges from the nose, and occasionally the accessory sinuses, throat, lungs and even vomitus. Rigid interpretation of the findings should seldom be made unless the interpreter is fairly certain that the material examined comes from the area in question. The presence of tubercle bacilli in a sputum is not in itself sufficient to indicate that we are dealing with a pulmonary sputum.

We have been able to show from cases of open pulmonary tuberculosis that the bacilli may be found in saliva, in water used for gargling the throat and even water used for rinsing the mouth. Such material would obviously be relatively free from albumin. Further, tuberculosis in a certain portion of a lung does not exclude other pathological conditions in different portions of the respiratory passages. One patient actually furnished in the same day a sputum free from tubercle bacilli and albumin and another containing a fairly large number of tubercle bacilli and 8 per cent albumin. Such conditions are probably rare, but we believe the possibilities they present offer satisfactory explanation for the low or absent albumin findings in exception to the general rule.

It cannot be said that there is any specific relation between tuberculosis and the presence of albumin in sputum, for albumin appears to be constantly present in pneumonia, pulmonary edema, and abscess of the lung as in abscesses of other parts of the body. Our findings indicate that albumin is present in the sputum of practically all cases of active pulmonary tuberculosis and that in over 80 per cent of these the amount is above 10 per cent by volume. In chronic rhinitis, pharyngitis, laryngitis, bronchitis and asthma albumin is generally absent or present only in traces. In acute conditions the findings are less constant, while the absence of albumin is the rule in all superficial inflammations of mucous membranes, abrasions or lacerations of the surface epithelium are occasional accompaniments to such cases and give rise to serous or bloody exudates that contain albumin in abundance. An absence of albumin we believe excludes active tuberculosis as the source of that particular sputum.

The presence of albumin *per se* therefore has no great diagnostic or prognostic value and it is only when quantitative determinations are considered together with other findings that the most valuable conclusions may be drawn.

It has been generally accepted that active tuberculosis in the various parts of the body gives rise to a lymphatic exudate and it has been customary among laboratory men to diagnose tuberculosis from effusions high in lymphocytes when coming from sources outside of the lung. The same principles, however, do not appear to have been generally applied to the study of sputa.

We believe that a predominance of lymphocytes together with high albumin justifies the diagnosis of tuberculosis in the absence of tubercle bacilli from a sputum exactly as well as do the same findings from a pleuritic exudate or spinal fluid. Either albumin or lymphocytosis, however, taken alone is unreliable and does not justify such a diagnosis.

The most valuable factor of the albuminous exudate as it occurs in active pulmonary tuberculosis is the fact that it is independent of the presence or absence of tubercle bacilli in the sputum. Consequently it is independent of whether the lesion is open or closed. We have therefore in the albumin determination an index to the activity of a lesion that appears before the tubercle bacilli are found and generally before secondary infection has taken place. Under such conditions a high albumin associated with a predominance of lymphocytes appears frequently and early and gives us a reliable basis for diagnosis. As a means of prognosis the quantity of albumin appears to be a direct index to the activity of the lesion. We have followed a considerable number of cases through their incipency and have had the good fortune also to follow some cases well into recovery. The findings on such cases indicate that albumin appears early before tubercle bacilli are present, diminishing and disappearing with the arrest of the process.

A Clinical Method for the Determination of Carbon Dioxide in Alveolar Air

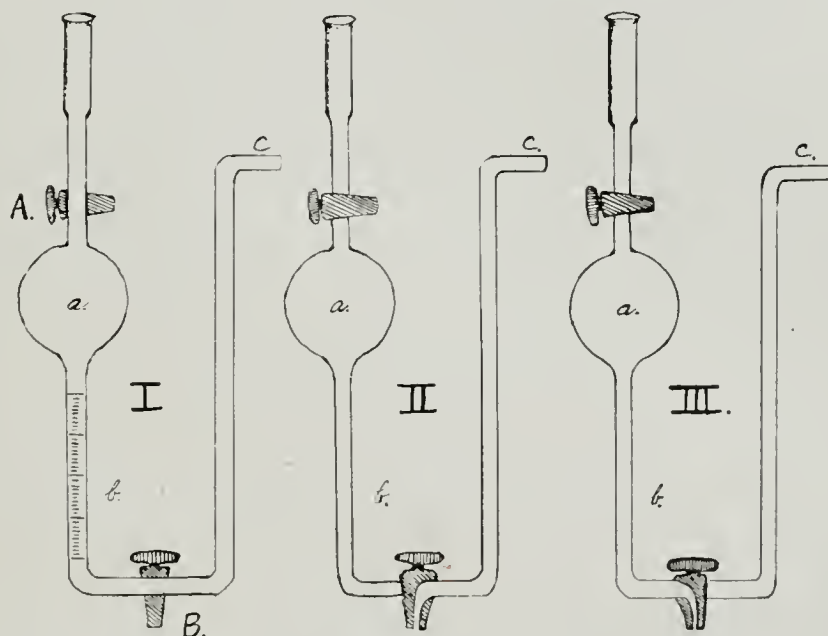
BY J. J. R. MACLEOD, M.B., CLEVELAND, OHIO.

THE percentage of CO_2 in the alveolar air corresponds to the tension of this gas in the blood, that is, to the amount of CO_2 which is in simple solution in the plasma (Haldane and Priestley, Krogh). Since this tension of CO_2 in the blood depends on the equilibrium between acids and bases therein contained, it will become less whenever an excessive amount of acids other than CO_2 is present, just as would be the case if we were to add some strong acid to a solution of bicarbonate. In both cases, after the immediate displacement of CO_2 by the stronger acid, a smaller amount of bicarbonate, and therefore of free CO_2 , would remain in the solution. When a subnormal percentage of CO_2 is present in the alveolar air, therefore, it indicates that there is an excess of fixed acids in the blood. *It indicates that a severe degree of acidosis exists*, and is a danger signal of impending coma. It has been known for long that very low alveolar CO_2 percentages are obtained in late stages of diabetes, but until recently the fact could not well be used for diagnostic purposes because of the

more or less difficult method (of Haldane) which had to be employed for the estimations. Fridericia¹ has recently furnished us with a sufficiently accurate and yet very simple method, which should now make estimations practicable in the clinic.

This method is as follows: The necessary apparatus consists of a U-shaped glass tube (see Fig. 1) with stopcocks A and B, as depicted in the accompanying figures. A is a simple stopcock, but B is three-way, as shown in Figs. II and III. Between these stopcocks the tube has a bulb blown on it so that the total capacity between A and B is exactly 100 c.c., the part of the tube below the bulb being graduated 1/10 cubic centimeters. A cylindrical vessel large enough to hold the apparatus and containing water at room temperature, an ordinary evaporating dish, a 20 per cent solution of KOH, a saturated solution of boric acid, and a rubber bulb fitting to the side tube C, are also required.

To make an estimation the apparatus is first of all rinsed out with the boric



acid solution (which absorbs practically no CO_2). The patient (who may be lying in bed) is told to take the end of the tube, C, in his mouth after an ordinary expiration and to blow out all the air he can through the apparatus, with A and B open as in Fig. I. When he has done this and before removing the apparatus from the mouth, the tap A is closed. The entire space in the apparatus is now filled with air which must have come from the alveoli, since the "dead space" of the lungs (i. e., the mouth, trachea and bronchi) has a capacity of not more than 130 c.c., whilst the volume of air, which even a weakened patient could blow out, measures at least 1,200-1,500 c.c. The apparatus is now placed for 5 minutes in the cylinder of water so as to cool it down to the temperature of the room, after which it is removed and the tap B turned into the position indicated in Fig. II. Between A and B we now have 100 c.c. of alveolar air at room temperature. The wider portion above stopcock A should alone be handled in removing the apparatus, so as not to warm the gas in the confined portion. (During the cooling process some of the air in the part of the tube

¹Fridericia, I. S.: Eine klinische Methode zur Bestimmung der Kohlensäurespannung in der Lungenluft, Berl. klin. Wchnschr., 1914, li, p. 1268.

between B and C will have been sucked into the graduated portion because of shrinkage, but this will not vitiate the analysis, because it also is alveolar air.)

It is now necessary to ascertain what proportion of this 100 c.c. of air is CO_2 . This is done as follows: Turn the stopcock B into the position in Fig. 11, place the side tube of the stopcock in an evaporating dish containing 20 per cent KOH, and by means of the rubber bulb suck this solution up to the bend of the tube C. Turn stopcock B so that it is completely closed, and after carefully removing the rubber bulb from C, again turn stopcock B as in Fig. 1, (but with A still closed) so that some KOH solution runs into the graduated portion of the apparatus. In doing this care must of course be taken that no air enters the graduated portion. Stopcock B is now turned as in Fig. 11 and the KOH solution in the free tube allowed to run out, after which this stopcock is turned off and the apparatus inverted several times so that the KOH may absorb all of the CO_2 in the confined air. The apparatus is now submersed in the cylinder of water up to stopcock A and B is turned, as in Fig. 11, so that water can run into the graduated tube. It will run in because a partial vacuum has been created on account of the absorption of CO_2 . After allowing time for temperature conditions to become equalized (5 minutes), the apparatus is now cautiously raised until the water inside and outside the graduated tube stands on the same level. Stopcock B is turned off and the apparatus removed and the graduation read at which the meniscus stands. The reading gives us directly the percentage of CO_2 in the alveolar air.

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ROY G. PEARCE, M.D.	-	-	CHICAGO
ROGER S. MORRIS, M.D.	-	-	CINCINNATI

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EDITORIALS

The Role of Fixed Tissue Cells in Protecting the Body Against Infection

IT will be remembered that the science of immunity, after being founded on the basic observations of Pasteur on active immunization, developed along two parallel lines,—one the investigation of the direct action of serum constituents on the invading bacteria and their products, the other centering upon the activities of phagocytic cells. These two schools have more recently met upon common ground in the recognition of cooperative activity between plasma and cells. Though it is generally not recognized, the idea that the serum antibodies might act upon the bacteria preparing them for phagocytosis was expressed by Metchnikoff himself early in his investigations.¹ However the firm establishment of this fact was postponed until its experimental proof by Denys and his collaborators, by Wright and by Neufeld. Since its recognition there has been a general tendency to look upon phagocytic immunity as the perfect mechanism for protection and there has been a suspicion that the reaction of serum antibodies and bacteria without the cooperation of phagocytic cells might even result in injury rather than defense by the liberation in such a process of toxic substances from the bacterial protein, substances analogous at least to those obtained by hydrolytic cleavage by Vaughan. In infections with such organisms as the cholera spirillum and the typhoid bacillus, it has been shown by Bordet, and more recently by Gay and Claypole, that immunized animals responded to

reinjection with a leucocytosis far in excess of that following the first injection into normal animals.

Although, therefore, the phagocytic functions of the mobile leucocytes have been very intensively studied, yet insufficient attention has been paid to the phagocytosis carried on by fixed tissue cells. We have had a vague understanding of such phagocytic processes in tuberculosis, leprosy, blastomycosis, rhinoscleroma, and possibly syphilis, but the difficulty of experimentation has been such that little progress has been made. It has been known that the cells of the liver and spleen, young connective tissues, the dust cells or alveolar² cells of the lung, and many other cellular elements not mobile in the circulation, could take up bacteria. It has been supposed that tubercle bacilli not digested within the polymorphonuclear leucocytes were destroyed by the giant cells appearing in tuberculous lesions, and the writer, with Cary, showed some years ago that rat leprosy bacilli not injured morphologically during prolonged sojourn within polymorphonuclear leucocytes were rapidly digested in spleen cells grown in vitro. Adami,³ moreover, as early as 1899 called attention to the fact that, during normal life, living bacteria may enter the portal circulation to be very largely destroyed in the liver, the lymphatic organs or the kidneys, a condition of which he speaks as *sub-infection*. However all this work has established no definite relationship between fixed-cell phagocytosis and resistance.

In a recent paper, which appears to us very important in its general significance, Kyes⁴ has demonstrated a remarkable parallelism between an instance of natural resistance and fixed-cell phagocytosis. He chose for his experiments the pneumococcus as the infecting agent and the pigeon as the naturally resistant animal. Apparently it is impossible to produce symptoms of disease in this animal with large doses of virulent pneumococci, and this has been hitherto regarded as largely dependent upon the high body temperature of pigeons. However Kyes's experiments seem to show that the temperature alone cannot explain the rapid disappearance of the bacteria when injected into this animal, since 96 hours' exposure at 43° C. will not destroy pneumococci, but, injected into the blood stream, several billions are taken up within ten minutes and are totally destroyed within 72 hours. Kyes's work does not imply, nor does he draw the conclusion, that the temperature has no protective value; in fact, it is not impossible that the complete and rapid destruction of the bacteria is made possible by the high temperature and the consequent failure of multiplication of those injected. However Kyes has discovered a mechanism of bacterial destruction which, though vaguely indicated in earlier work, has never before been so thoroughly recognized. He has found that pneumococci injected into the general blood stream in pigeons are rapidly localized in the liver and spleen and then are taken up within the hemophagic cells, which, localized both in the liver and spleen, are supposed normally to serve the function of red blood cell destruction. The rapid destruction of the pneumococci by these cells is shown definitely by Kyes's work to constitute a very important factor in freeing the animal body of the invaders.

This work is remarkably similar in tendency to the work of Bull⁵ of the Rockefeller Institute on the removal of typhoid bacilli injected into normal rabbits, in which Bull has for the first time demonstrated a possible immuno-

logical function for agglutinins. It appears that the injected bacilli rapidly disappear from the circulating blood. They are intravascularly agglutinated and the clumped bacilli are taken up by polymorphonuclear leucocytes and other cells in the liver and the spleen. A similar destruction of pneumococci in the liver and spleen has been shown to occur by Berry and Melick,⁶ after intraperitoneal injection.

These observations have fortified the rapidly growing impression that, in the removal of bacteria from animal bodies, the fixed tissue cells may play a much larger part than hitherto suspected. They are, furthermore, gradually strengthening our belief that very little actual importance is to be attached to the bactericidal processes in the serum, which, indeed, may play a very secondary part in protection.

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—H. Z.

The Epiphysis (The Pineal Gland)

THE epiphysis is formed at the caudal end of the diencephalic roof. It consists originally of a thin ependymal diverticulum between the posterior commissure and the habenular commissure. Subsequently its walls are thickened and incorporate some of the adjacent vascular mesoderm to form the adult organ (Streeter¹). The epiphysis is therefore essentially an epiblastic organ, a fact which makes it difficult to account for the further fact that the most frequently encountered tumors affecting it are teratomas.

The evidence which connects the pineal gland with disturbances of growth and function of the body is incomplete, and is even less satisfactory than that concerning the thymus.

It is probable that the inaccessibility of the pineal has been the reason for the fact that very little experimental work has been carried out in connection with this gland, and for the unsatisfactory results of experiments, and that so little is known of its possible function. Up to within very recent years all that has been suspected of it has been based almost alone upon clinical data. It is true that since 1910 several investigators have made attempts to gain experimental data, but the great difficulties of operative procedures were not surmounted until 1915 when Dandy perfected a technic which permitted a quick complete removal after a very brief operation. It is perhaps unfair to a gland to draw conclusions regarding its place in the economy when the operative procedure alone produces a death rate of 75 out of 95 animals (Exner and Boese), or, in another series, of 12 out of 15 (Sarteschi).

The clinical data which have suggested that pineal abnormalities are associated with sexual precocity and adipose overgrowth, are derived chiefly from

the cases of Heubner (Oestreich and Slawyk), Ogle, Machell, Fränkl-Hochwart, and Raymond and Claude, in which the pineal was the seat of a teratoma. The experimental data emanate from the feeding experiments of McCord, Dana, Berkeley, Goddard, Goddard and Cornell. The clinical data have come as a rule from cases in which the pineal has been involved in a tumor growth, and therefore they are unsatisfactory, because under such circumstances one does not know to what extent other neighboring organs are modified, directly or indirectly by the growth. It is an interesting fact that the tumors in such cases have been almost without exception teratomas.

Since Heubner's report of his case in which a teratoma of the pineal was found at autopsy by Oestreich and Slawyk,² the attention of clinicians was focused upon the possibility that cases showing sexual precocity with or without similarly pronounced adiposity, were the subjects of pineal lesions. Later Marburg³ from a study of the cases reported in the literature and his own (about forty in all), scheduled his conception regarding hypopinealism, hyperpinealism and apinealism, by which he introduced what he believed to be pineal complexes, by clinical study of which he could say that hypopinealism was associated with sexual precocity, obesity with hyperpinealism, and cachexia with apinealism. This seemed to indicate that hypophyseal lesions and pineal lesions tended in opposite directions, for while Fröhlich's syndromes might result for underactivity of the pituitary, an indistinguishable syndrome could follow overactivity of the pineal.

Marburg believed that the pineal was a functionally active gland only during the early years of life, and that during this period, it inhibited the development of the sexual side of growth, and that with its gradual involution during youth, its inhibitions were also gradually lost, until at the period of maturity, the pineal no longer had a necessary part to play in the economy. It seemed possible, following this conception, that if, before involution was complete, the pineal was destroyed, sexual and somatic development would go on apace relieved of the normal pineal inhibitions. It should be remembered, as Dandy says, that the whole structure of Marburg's hypothesis was based upon clinical and anatomical studies only, and not upon experimental work.

In 1910, experimental attempts to extirpate the gland were undertaken by Exner and Boese⁴ who were able to discover no changes after incomplete or complete removal. Other experimental work has contradicted these results (Sarteschi, Foà). However the operative experimental results are no more contradicting than those based upon treatment with gland extracts or feeding of the gland substance. In the operative examples, however, the value of the work is modified by the technical difficulties which were not surmounted.

No less contradictory than the results of operative experiments have been those of feeding. McCord⁵ fed guinea-pigs, chickens and dogs with pineal materials and believed that this treatment produced precocious sexual changes, accompanied by an increase in weight, and also accentuation of the mental condition in the direction of precocity, and Dana and Berkeley⁶ reported weight increases above the normal in guinea-pigs, rabbits and kittens. In fifty children who were given pineal injections, however, growth in height and weight was

less marked than in controls, but there was a certain mental effect which was analogous to that which McCord had recorded in his puppies.

Dandy⁷ summarizes the results of experimental work up to the time of his own report as follows: "Adiposity may result by feeding pineal extract (McCord, Dana, and Berkeley) or by complete or partial removal of the pineal (Sarteschi). Sexual and somatic precocity may result from feeding pineal extracts (McCord, Dana, and Berkeley), or from partial or complete removal of the pineal body (Foà, Sarteschi), or nothing may result from its partial or complete destruction (Exner and Boese)."

It was with the object of shedding a little light into the shady pineal region that Dandy perfected and put into practice an operative procedure which is free from objection. Little trauma is produced upon the brain. Little or no hemorrhage results, and the pineal can be removed completely. He has made a series of studies upon young puppies, from ten days to three weeks old, and also in several adult dogs of both sexes. His results are striking and confirm those of Exner and Boese, in that no evidence was obtained of any somatic, sexual or mental effects in the direction of inferiority or superiority. The conclusion seems to be, and Dandy reaches it, that the pineal is not essential to life and seems to have no effect upon the well-being of animals.

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—P. G. W.

Some Recent Contributions to Our Knowledge Concerning the Metabolism of Fat

THE mechanical view of the absorption of fat by the intestinal epithelium has now been entirely replaced by the chemical view. According to this the fat is split by the agency of the "lipase" of the pancreatic juice in the upper levels of the intestine into fatty acid and glycerine. The fatty acid becomes combined either with alkali or with the bile salts. Unlike the fatty acids themselves the soap and the compound of bile salts with fatty acids are soluble in the water of the intestinal contents. The dissolved glycerine and fatty acid are separately absorbed into the intestinal epithelium, in the protoplasm of which they re-combine to form neutral fat. The neutral fat thus produced gradually finds its way from the epithelial cells to the central lacteals of the villi, and thence by way of the thoracic duct, into the blood stream.

A most important application of this view is that substances other than fat will not be absorbed by the intestinal epithelium, however like fat they may be in their physical properties (i. e., emulsify well with dilute alkali, dissolve in the fat solvents, melt at the body temperature). Petroleum emulsion, for example, according to physical and microscopic examination, may be in every re-

gard identical with an emulsion of neutral fat, and yet if a compound emulsion composed partly of neutral fat and partly of some petroleum be administered to an animal, it will be found that all of the neutral fat is absorbed, but none of the petroleum. This can be demonstrated either by examination of the feces or of the lymph flowing from the thoracic duct during the absorption of the emulsion. Experiments of this type were performed several years ago by Henriques and Hansen, but the results, as stated above, were challenged by Bradley, who claimed to find some of the petroleum absorbed. It has recently been shown by Bloor,^{1, 2} however, that the former investigators were correct. Obviously such an experimental result once and for all disproves the mechanistic theory of fat absorption.

Not only petroleums, but fat-like substances which are incapable of being attacked by the lipase of pancreatic juice, also fail to be absorbed, for example, wool fat, which is a compound of cholesterol and fatty acid. Now, one characteristic of all these substances that are not absorbed is that, although physically, and in many regards chemically identical with fat, they fail to be reduced to a water-soluble condition in the intestine. They are soluble in fat and in fat-solvents, but they are incapable of saponification in the intestine.

It might be objected to the above conclusions that, although undetectable, there is really some essential physical difference between emulsified fat and emulsified hydrocarbon. In order entirely to prove the case for the chemical theory, it is necessary to feed a neutral fat possessing some characteristic that depends on the manner of union existing between the fatty acid and glycerine, and then to see whether it appears in an unchanged condition in the thoracic duct. If it does so, the fat must have been absorbed through the intestinal epithelium in an unbroken, unsaponified, condition, for it is unlikely, in the resynthesis which occurs in the intestinal epithelium, that the fatty acid molecules would re-combine with the glycerine molecules in exactly the same manner as before.

There are, however, but very few qualities of neutral fat, apart from those of the fatty acids which compose them, by which they can be characterized. The most likely one is that of optical activity. None of the ordinary fats is optically active, although from chemical considerations it is quite conceivable that they might become so. In order to obtain such a fat Bloor^{4, 5} conducted numerous experiments with the esters of stearic acid. He prepared an optically active mannitan di-stearate, but found it to have a very high melting point and to be only half as digestible as the ordinary fats. Its absorption was too slow and unsatisfactory to make it suitable for the above purposes. He, therefore, proceeded to prepare the di-ester of isomannitan with lauric acid, and he found the resulting compounds to be as well-absorbed as ordinary fat, and yet to possess very marked dextro-rotatory power, which of course they lose on saponification. This fat seemed suitable therefore for testing the above question. In a series of experiments, Bloor fed isomannid-di-laurate and by examination of the neutral fat present in the chyle flowing from the thoracic duct, found no evidence of the dextro-rotatory fat. This result confirms previous work by Frank, who found that the ethyl esters of fatty acids are not absorbed unchanged. The results of both groups of workers emphasize the probability that readily

saponifiable fatty acid esters do not escape saponification under the favorable conditions of the normal intestine. In other words, had the fats been absorbed unchanged, as would be required by the mechanistic theory of fat absorption, they would have appeared in the chyle in optically active condition.

These most important conclusions lead us to inquire as to the reason for this change in fat during its absorption. It cannot be for the purpose of preventing the absorption of undesirable fatty substances, such as the petroleum hydrocarbons or the wool fats, because such substances are so rarely present in our food. It is most probable that the breakdown and resynthesis of neutral fat occurs for the same reason that similar processes occur during the absorption and assimilation of protein. It will be remembered that protein is entirely disintegrated in the intestine into its so-called building stones. These are absorbed separately into the blood, which carries them to the tissues, in which they become resynthesized to form the body protein. And so it appears to be in the case of fats. The process, in other words, permits of the rearrangement of fatty acid molecules, as a result of which the newly formed fat is more adaptable for use in the organism. It comes to be more like the characteristic fat of the animal. There may be another reason for the process. It will be remembered that lecithins, which constitute the most important of the fatty substances of the cell itself, are mixed glycerides, that is to say, are compounds containing a variety of fatty acids. The rearrangement of the molecule of neutral fat which occurs during absorption may be the first step in the transformation of fat into lecithin.

In order to throw further light on the question, Bloor has performed a number of interesting experiments in which he compared the chemical properties of fats before and after their absorption. The criteria which he took were melting point, iodine value and mean molecular weight; the melting point represents the solidity of the fat, and the iodine value, its degree of unsaturation, that is, the number of double links that exist in the fatty acid chain.* Bloor found that during absorption very considerable changes in these two characteristics occur. For example, when fat with high melting point and low iodine value was fed, the fat in the thoracic lymph was found to give fat of distinctly lower melting point and higher iodine value. When fat with a low melting point and high iodine value was fed, the reverse change occurred, for the melting point of the thoracic lymph fat was higher and the iodine value lower. These results in the first case could be explained as due to the addition of oleic acid to the fat during its synthesis in the intestinal epithelium, and in the second case to the addition of some saturated fatty acid. When a fat was fed consisting mainly of glyceride of saturated fatty acid, but having a low melting point, the addition of oleic acid was still found to occur, as judged from the iodine value. This leads us to conclude that the change which occurs is not merely in order that the melting point of the absorbed fat may be lowered, but also for some chemical reason. In a fourth series of experiments, a lowering of iodine value occurred after feeding with a fat, namely, cod-liver oil, which already contained a high percentage of glyceride of very unsaturated fatty acid.

*These estimations were really made on the isolated fatty acids.

Evidently then the intestine possesses the power of modifying the composition of fat during its absorption, and this modification is apparently of such a nature that it causes a change toward the production of a uniform chyle fat, presumably the characteristic of the animal body. It remains of interest in this connection to consider the source of the oleic acid or of the saturated acid required for this synthesis. Bloor quotes work by other observers to show that the changes are probably greater than could be produced by admixture of the absorbed fat with the fat present in the normal fasting chyle.

Important work has recently been done concerning the fat present in the blood. Normally the blood contains only a small percentage of fat, but after a fatty meal it may contain so large an amount that the fat actually rises to the surface of the blood like a cream. By means of the ultra-microscope, examination of the blood in the dark field after a rich meal reveals the presence of glancing particles, the so-called "fat-dust." These particles are most abundant about six hours after the meal has been taken, and they gradually disappear again by the twelfth hour. These facts have been known for some time, but it has been impossible, either on account of the large quantities of blood required for a chemical examination or because of the inability to estimate the amount of fat from the density of the "fat-dust," to follow with any great degree of accuracy the exact changes that take place in the fat of the blood. It is, therefore, a most important contribution that Bloor should have succeeded in elaborating a method by which the fat-content of the blood can be fairly accurately estimated, using only small quantities of blood. This permits of a continuous series of observations over a considerable period. In Bloor's method,⁸ the fat is extracted from the blood by an alcohol-ether mixture with moderate heat. An aliquot portion of the filtrate is evaporated in the presence of sodium ethylate; this saponifies the fat. The residue consisting of soap is well washed and is then treated with hydrochloric acid so as to precipitate the fatty acid. The density of the precipitate thus produced is compared in an optical apparatus, called a nephelometer, with a standard solution of two milligrams of oleic acid, treated in the same way. The lecithin and cholesterol may also be estimated in the same blood extract.

For lecithin⁹ the above extract of blood, after the removal of the alcohol and ether, is digested by heating with concentrated HNO_3 and H_2SO_4 . This decomposes the lecithin, liberating the phosphorus, a solution of the resulting ash being rendered faintly alkaline to phenolphthalein and then slowly added to a silver nitrate solution. The density of the precipitate thus produced is compared in the nephelometer with a precipitate produced in the same amount of silver nitrate by adding to it a standard phosphoric acid solution.

For cholesterol an aliquot portion of the above extract is saponified with sodium ethylate and then saturated with chloroform; the chloroform extract is mixed with acetic anhydride and H_2SO_4 (con.) until the bluish color is fully developed (Liebermann reaction), the intensity of which is then compared in a colorimeter with that obtained by similar treatment from a standard cholesterol solution.

By the use of these methods Bloor has made a careful study of the varia-

tion in the fat content of the blood under normal conditions in dogs. He has found⁶ that the percentage of fat is remarkably constant under normal conditions, which, he says, probably indicates that in man also this is the case. The examination of the blood after a fatty meal showed that the increase in fat content began in about an hour, and reached its maximum in about six. This increase was not found in animals in which the thoracic duct had been ligated. Although this result would seem to contradict the view which some have held, that part of the fat which cannot be accounted for in the thoracic duct lymph is absorbed by way of the portal vein, Bloor does not commit himself on this point, but merely states the result and calls attention to previous work by d'Errico to the effect that the fat content of the portal blood is always higher than that of the jugular.

Very interesting results were obtained following the intravenous injection of emulsions of oil, either the so-called casein emulsion or colloidal suspensions. Up to a dose of 0.4 gm. per kilogram of body weight—which by calculation would suffice to raise the fat content of the blood by 100 per cent—these caused no increase in fat content. In order to explain this disappearance of fat, it might be imagined that the injected fat particles formed emboli in the smaller capillaries. Against such a view however is the fact that the particles of fat in these emulsions were one-half to one-seventh the size of a red corpuscle. Although this argument is no doubt of some weight, it ought to be remembered that the physical condition of these fine fat globules is not the same as that of the red blood corpuscle. Their surface condition may be such that they readily agglutinate so as to form small masses which may stick at the branching of the smaller arterioles and capillaries. Bloor himself suggests that the injected fat may be stored, possibly in the liver, since the fat in this organ, as we shall see later, increases in similar experiments. When twice the above quantity of fat was fed in the form of egg-yolk fat, some of it persisted in the blood for several hours. This increase may have been due to the flooding of the temporary storehouse with fat, or, more probably, to a retarding influence which lecithin may have on fat assimilation. That lecithin itself persists in the blood for a long time after intravenous injection had previously been demonstrated by Nerking.

A careful study was also made of the blood-fat during fasting. No increase was found unless the animal, by special feeding, had been stuffed with excess of fat prior to the fasting period. Narcotics were found to produce an increase in blood-fat, but ether produced this increase during the narcosis, whereas morphine and chloroform did not do so until after recovery. The explanation given for the ether effect is that a mixture of blood and ether has higher solvent power for fat than blood alone. The explanation for the chloroform and morphine effects is that a certain amount of breakdown of the tissue cells, in which lipins are set free, supervenes upon the action of these narcotics. At the conclusion of this paper Bloor suggests that there may be two places in the body in which surplus fat is stored: (1) a temporary storehouse, from which the fat is readily taken up and liberated, but which is of limited capacity, and (2) a more permanent storehouse, into which the fat is slowly taken up, but the capacity of which is very much greater. The temporary storehouse may

be under the control of some quickly acting fat-regulating mechanism, like that of the glycogenic function of the liver.

Most suggestive further observations by the same worker have recently appeared.⁷ By comparison of the fat acid, lecithin, and cholesterol content of blood during fat absorption, it has been found that there is a steady but very variable increase in fatty acid, accompanied by no variation in cholesterol, but with an increase in lecithin, which varies from 10-35 per cent, but does not run strictly parallel with the fatty acid increase. It is pointed out that this increase in lecithin may represent that part of the absorbed fat which is intended for immediate use in the tissues. The more or less independent increase in lecithin observed in these experiments is of significance in connection with the fact, which has recently been established, that in many pathological conditions of so-called lipemia the increase does not affect the fats of the blood but rather the lipoids (i. e., lecithin and cholesterolin). In yet unpublished work communicated to the Biochemical Society in December last, Bloor has found that separate analysis of blood plasma and whole blood shows the increase of lecithin to be much more marked in the corpuscles than in the plasma, whereas the fatty acid increase is confined to the plasma.

Besides the temporary and permanent fat storehouses (the so-called fat depots), there are two other places in the animal body where fat exists, namely, the protoplasm of the tissue cells and the liver. The immediate function of the fat in each of these places is not the same. In the depots, such as the subcutaneous tissue and the mesentery, the fat exists purely for storage purposes; in the protoplasm of the tissue cells it exists for the purpose of being oxidized to liberate the stored energy; and in the liver it appears to exist for the purpose of being prepared for combustion in the tissues. It is of great practical importance, therefore, that chemical methods should have been found which enable us to distinguish the characteristic properties of each of these types of fat. The chemical properties which have been used for this purpose are the iodine value and the percentage yield of fatty acid of the fat. The iodine value is a measure of the unsaturated fatty acids present and is highest in the tissue fat, lowest in the depot fat, and it usually occupies an intermediate but variable position in the case of liver fat. The percentage of fatty acid indicates the size of the molecule into which the fatty acid is built, being high in the case of the simple glycerides (neutral fat) and low in the case of the phospho-lipins (lecithin, etc.). This percentage is lowest in the tissue fat, highest in the depot fat, and like the I-value it usually occupies an intermediate but variable position in the fat of the liver. These chemical differences between the depot, the tissue and the liver fat have made it perfectly clear that the ordinary history of fat consists in its deposition in the depots and the subsequent withdrawal of this fat for combustion purposes in the tissues, an intermediate stage, however, being transportation to the liver, where it is prepared for tissue consumption. The nature of this preparation of fat which occurs in the liver has been very carefully and thoroughly studied by J. B. Leathes and his pupils. It is work the initial stages of which were completed several years ago, and to which contributions are every now and then being made.

Of the recent contributions those of Coope and Mottram deserve especial mention.^{11, 12, 13} These authors have observed that infiltration of the liver with fat occurs during pregnancy and early lactation in laboratory animals, particularly in the rabbit. They point out that all evidence, so far, that fat may migrate from the depots to the liver has been furnished by observations on animals under abnormal conditions; for example, either in phosphorus or phlorhizin poisoning in laboratory animals, or as a result of disease in man, as in cyclical vomiting, diabetes, etc. The above authors found that the fatty acid deposited in the liver in late pregnancy gives an I-value which lies nearer to that of the mesenteric fatty acid than is the case in normal animals. Mottram concludes that "wherever . . . there is abundant fat metabolism, the liver is found to be infiltrated with fats, presumably to be handed on elsewhere when worked up."¹³ It is interesting that the fetus is greedy of unsaturated fatty acids.

A practical clinical application of the above work is that fats will be more readily utilized by the body when they contain a high percentage of unsaturated fatty acids. It is probably for this reason that Norwegian cod-liver oil is of such undoubted nutritive value. It is much more so than Newfoundland cod-liver oil because in the preparation of this variety oxidation occurs, thus making it no longer unsaturated. Fish oils in general are more unsaturated than other animal oils, and are for this reason more nutritious.

The high percentage of unsaturated fatty acid in the liver as compared with that in the depots need not, however, *necessarily* indicate that the liver has produced the desaturation, for it might be that his viscus has high attractive power for unsaturated acids that is to say, that unsaturated acids circulating in the organism are especially appropriated by the liver cells. In order to test the hypothesis that the process of desaturation does really go on in the liver, Raper¹⁰ after trying various methods studied the I-value of the volatile fatty acids of the liver after feeding or injecting animals with cocoanut oil. This oil is volatile in steam, the distillate being saturated, that is to say, having no iodine value. Although such a distillate of normal liver has a certain iodine value of its own, it was hoped that after feeding cocoanut oil a change might occur in the distillate from the liver, indicating that desaturation of the absorbed or injected fatty acids had occurred. It was found in some experiments that the volatile oils obtained from the liver, after giving the cocoanut oil, absorbed more iodine than those from a normal liver.

One interesting outcome of this research was the fact that in anesthetized animals cocoanut oil, placed in the intestine along with glycerine and bile salts, was absorbed to the extent of 30 per cent by the liver. When given in fine emulsion intravenously from 25 to 60 per cent of it was found in the liver. This retention of the oil by the liver may of course be due in part at least to the high and sudden concentration of the oil in the blood, and it may be partly due to the anesthetic.

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—J. J. R. M.

Intraspinal Use of Phenolsulphonphthalein

THE most convincing investigation of hydrocephalus is that of Dandy and Blackfan.¹ Their work has frequently been quoted as experimental corroboration of the rationale of intraspinal medication.²

The importance of their conclusions lies, first, in their demonstration of the feasibility of injecting phenolsulphonphthalein into the spinal canal. Long experience with this drug in functional kidney tests has proved it inert and capable of accurate quantitative detection. Secondly, they use pressure never over normal. Lastly, they were able to completely verify their animal experiments on human beings.

They injected 6 mg. of the drug in 1 c.c. of water—a neutral solution, of course. In normals, when injection was made into the cisterna magna, they could promptly recover it from the ventricles, and when injected into the ventricles, from the spinal subarachnoid. Without pressure they could inject the whole spinal and cerebral subarachnoid with insoluble granules. By experiments on normal human beings, they thus established three standards for phenolsulphonphthalein: (a) the time (1-3 minutes) of appearance of the drug in the spinal fluid after injection into the ventricles; (b) the time (10-12 minutes) and the rate (12-20 per cent in two hours) of excretion in the urine after ventricular injection; and (c) the time (6-8 minutes) and the rate (35-60 per cent) of excretion after subarachnoid injection.

In nine hydrocephalic children they were able to show that after spinal injection the drug appeared in the urine in normal time and amount, but that after ventricular injection it did not appear in the urine at all for the reason that it did not pass out into the subarachnoid space. Seven of these cases came to autopsy and in all the experimental findings were confirmed by demonstration of lesions mechanically obstructing the iter or foramina.

¹Dandy and Blackfan: *Am. Jour. Dis. of Child.*, Dec., 1914

²Ogilvie, H. S.: *Jour. A. M. A.*, Nov. 28, 1914, and Swift, J.: *Jour. A. M. A.*, July 17, 1915.

Four other cases of hydrocephalus showed a normal passage of fluid from the ventricles to the subarachnoid, but here the absorption from the later space was delayed, averaging only 10 per cent in two hours. As the renal elimination was proved normal in all these, the assumption is justified that there is a type of hydrocephalus dependent solely on insufficient elimination from the subarachnoid. No autopsies were obtained in these cases.

In discussing the possible egress of spinal fluid through the pacchionian granulations, Dandy and Blackfan say—"Any evidence of the passage of fluid through the pacchionian granulations during life would be very difficult, if not impossible, to obtain. Consequently all proof is dependent on postmortem injections. In such a condition it is possible to force fluid through the pacchionian granulations with very high pressure." It seems strange that they should not have been familiar with the work of Cushing and his co-workers,³ published only three months before their own. As a matter of fact, using living animals and only moderate pressures (180 mm. of water), Cushing did the very thing in question. True solutions—potassium ferrocyanide and iron-ammonium-citrate were precipitated after death by fixation in acid formalin and by the Prussian blue reaction were readily detected in the very act of passing through the walls of the pacchionian granulations into the sinuses.

As mentioned above, the work of Dandy and Blackfan has been quoted in favor of intraspinal therapy. If one were treating a purely luetic meningitis, one would be content with diffusion of his drug through the subarachnoid and their work substantiates the possibility of that. In endarteritis or general paralysis however the pericapillary and perineuronal spaces would have to be reached. Dandy and Blackfan did not secure such an injection. But in the work of Cushing the evidence wanted may be found, for, using pressures of 50 mm. of mercury, he secured a perivascular, perineuronal and, selectively, for some large cortical motor cells, an intracellular injection! The mere fact that phenolsulphonphthalein appears in the urine obviously supposes its passage through the blood vessels, but neither Dandy and Blackfan, nor Cushing and his co-workers believe in an absorption of spinal fluid by the cerebral end-vessels. Only in that way could the drug bathe the end-vessels and nerve cells in passing out into the circulation. To settle the question conclusively we must wait until someone has the good fortune to secure an autopsy not long after an intraspinal injection and demonstrates the presence of the drug in the structures we wish to reach. It is possible that the drug is absorbed from the subarachnoid without penetrating the parenchyma of the brain.

Other conclusions of Dandy and Blackfan are of minor interest confirming fairly established theories; i. e., experimental ligation of the vena Galeni magna produces hydrocephalus if it is made high enough to preclude collateral circulation; experimental hydrocephalus is less severe if the choroids are partly ligated before the iter is mechanically obstructed; venous congestion increases spinal fluid secretion; drugs and glandular extracts produce little effect; substances in solution in the blood and formed elements do not pass into the spinal fluid; in the dog, the fluid is restored every four or six hours; the lymphatics

³Cushing, Weed, and Wegefarrth: *Jour. Med. Research*, Sept., 1914.

play a very minor role in the escape of fluid; there is no evidence of a current in the spinal fluid; the fluid passes out along the sheath of only the first, second and fifth cranial nerves; the probable cause of hydrocephalus after operations for meningocele is limitation of the excreting surface.

The harmlessness and utility of phenolsulphonphthalein injections in the human is the most valuable contribution of the above research. It would no doubt be of great value to other cases with symptoms of intracranial tension—luetic meningitis, delirium tremens, etc.

—C. E. Kieley, per P. G. W.

Cancer and X-Rays

IN 1912 Murphy¹ showed that an avian embryo has no defensive mechanism against the growth of tissues from a foreign species. He later showed² that tumor tissue from the rat could be transplanted in avian embryos for indefinite periods. Also he showed³ that at about the time of hatching of an avian organism, a defensive mechanism developed which produced quick destruction of any foreign tissue. These observations were exceedingly interesting because they showed that heteroplastic grafting was possible providing the proper environment was furnished the graft. In these experiments the only morphologic facts observed were that in the young embryo there was no round cell infiltration about the foreign tissue, and that at about the time of hatching such an infiltration became noticeable. The only difference that could be discovered at the two periods was a difference involving the lymphocytes. With this in mind cultures of rat sarcoma in chicken plasma were prepared and to these were added bits of chicken tissues.⁴ The sarcoma tissue grew well in plasma alone, and in cultures containing adult chicken connective tissue, kidney, and liver. When, however, chicken spleen was added to a culture, growth of the sarcoma tissue stopped. Bone marrow gave the same result. These experiments were repeated using chicken embryos in the shell, with the same results, except that bone marrow proved to be less effective than the spleen in inhibiting growth. Still later Murphy⁵ showed that if an adult animal was deprived of a large amount of its lymphoid system by means of small repeated doses of x-ray, it no longer resisted the growth of heterologous tissue. The tissue cells of a foreign species will grow actively till such a time as the depleted system of the animal is well advanced in regeneration. This observation served to point emphatically to the role of the lymphoid tissue in tissue immunity. Murphy and Morton⁶ thereupon studied the blood picture in mice and were able to observe that in naturally immune animals the mononuclear count rose immediately after inoculation with tumor material, while in susceptible mice this did not happen. They showed that resistance to transplanted tumor tissue is accompanied by a marked lymphocytosis. To test the suggestion that the lymphocytes were indeed the active agents in this immunity, they thereupon used the method of Murphy and Ellis⁷ for destroying the major portion of the lymphoid system. They used the Coolidge tube, 10 milliamperes, 3 inch spark gap and an exposure of 1-2 minutes for several consecutive days, and

produced therewith a marked reduction of circulating lymphocytes. These x-rayed animals were then given an immunizing dose of blood, and were inoculated with tumor tissue, and compared with an immune series and controls. In the rayed series the incidence of growth was the same as in the controls, but the rate of growth was more rapid.

Murphy and Morton have also observed⁸ that after depletion of the lymphoid system by x-ray, regeneration will commence after a time and progress actively to a point where there is actually an overproduction of lymphoid cells. They also noted that by using a very small dosage of rays they could produce stimulation of the lymphoid structure. This naturally suggested a study of the therapeutic effects of the x-rays, and it was made in such a manner as to indicate the differences between the effects of the rays upon the animal as a whole, upon the tumor in it, and upon the tumor outside the body. To produce the necessary conditions they took mice in which cancers were growing and from them they removed the tumors temporarily while they gave stimulating doses of x-rays to the hosts, using the Coolidge tube. Immediately after treatment the cancers were grafted back into their hosts. In 50 per cent of these animals there was a complete immunity against the grafts. As controls they used animals affected in exactly the same manner as in the former group, and these were treated exactly as were the others except that the raying was omitted. The cancers were removed and then grafted back again. In 28 of 29 animals so treated the grafts grew progressively. In a third group (10 animals) the tumors were removed, treated with rays in the same dosage used previously on the animals, and then grafted back. In 100 per cent of these the grafts took and grew.

In this series of researches, there seems to be good experimental evidence that immunity to tumors (some at least) in mice is closely associated with the activities of the lymphoid tissues of the animals; and that the x-ray, given in proper amounts, may stimulate these lymphoid tissues and so render the animal resistant to tumor growth, or, in heavy doses, may destroy, temporarily, the lymphoid tissues, and so decrease resistance. With the ever increasing use of x-rays (to say nothing of radium) in the treatment of tumors in human beings, it would be of value to study the effects of treatments upon the tumor itself and upon the blood picture of the patient. It seems that a possibility exists that a differential blood count may be of very distinct value in indicating the course the treatments should follow. It would be exceedingly interesting to know whether or not the lymphocyte count can be made to rise or fall by x-ray treatments, and whether or not these fluctuations are related in any way to the therapeutic effects upon the tumors.

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The Presence of Eosinophile Cells in Pleural Exudates

INTEREST in the cytology of exudates in the serous cavities in the past has centered chiefly in the lymphocytes, endothelial cells and neutrophilic leukocytes. Evidence is accumulating, however, which shows that an important role is played by the eosinophiles at times.

E. Rist and de Pfeffel¹ have found, in a study of the exudates which follow in the course of artificial pneumothorax, that eosinophiles and mast cells are often conspicuous. The cytologic formula varies, moreover, with the duration of the exudate.

In exudates of recent formation, the authors find that eosinophilic leukocytes predominate, though they constantly find some mast cells. At times, they have found that these two varieties of leukocytes alone are present in the fluid. But, if the exudate persists, lymphocytes begin to collect in the fluid, few at first, though later they may predominate. At the time when the fluid shows an excess of eosinophiles and mast cells, the blood may contain 5 to 6 per cent of the former and 2 per cent of the latter.

In cases of spontaneous pneumothorax, Rist and de Pfeffel have found fluids containing eosinophiles and mast cells without neutrophiles, though more often neutrophiles are greatly in the majority.

Occasionally, they have seen the eosinophile mast-cell picture in the earliest stages of tuberculous pleurisy; soon, however, the usual lymphocytosis replaces it.

In the pleural effusions complicating lobar pneumonia, eosinophiles have been conspicuous in eight cases, according to S. Bayne-Jones,² who reports a case. Mast cells were also present in his case. Among the other conditions with which pleural eosinophilia has been found, according to Bayne-Jones' review of the literature, are trauma, sepsis, typhoid fever, syphilis, polyarthritis, nephritis, pulmonary gangrene, hemorrhagic infarct of the lung, endothelioma, septic endocarditis, gonococcal sepsis, myocarditis, cardiac insufficiency, puerperal sepsis, neoplasm, influenza.

The list of conditions is chaotic and the underlying cause or causes of the accumulation of eosinophilic and basophilic leukocytes do not seem evident. This only indicates the need of more systematic study of the cytology of exudates. Schwarz³ in 1914 was able to find but 68 cases in the literature, in which exudates showed an eosinophilia. Bayne-Jones, however, believes the phenomenon is of much more frequent occurrence than this would seem to indicate.

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—R. S. M.

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ORIGINAL ARTICLES

EXPERIMENTAL CLOUDY SWELLING OF THE KIDNEY IN THE RABBIT.*

BY W. RAY SHANNON, MINNEAPOLIS, MINN.

VIRCHOW¹ introduced the term "cloudy swelling" ("trübe Schwellung") in 1858 to describe a condition often found in the parenchymatous organs, such as the liver and kidney, in which the organ is swollen and light colored. It frequently presents a cooked appearance. When examined under the microscope in the fresh state, the cells are seen to be opaque, due to the presence of small albuminous granules. These granules may almost entirely hide the nucleus. The gross picture is due to the presence of these albuminous granules within the cells.

This classical description has been adopted with minor modifications by the majority of observers since Virchow's time. It has been copied into practically all of the modern text books of pathology.

Many pathologists often make a diagnosis of cloudy swelling upon the gross examination alone. But, if such diagnoses are checked by a microscopic study, it will frequently be found that there is no increase of albuminous granules. Turbidity and swelling of an organ are not always associated with increase of albuminous granules. On the other hand there may be a marked increase of albuminous granules in the cells without the presence of turbidity or swelling of the organ. It is, therefore, evident that cloudy swelling as commonly described includes more than one type of pathologic process. No important advance can be made until the several phenomena have been studied separately. With this in mind, I have undertaken the study of experimental cloudy swelling of the kidney, paying particular attention to the albuminous granules.

*From the Department of Pathology and Bacteriology, University of Minnesota.

*I wish to thank Dr. E. T. Bell, at whose suggestion and under whose supervision this work was carried out, for his helpful suggestions and enthusiastic interest. I am indebted to Mr. A. Lundquist for assistance in technic.

Cohnheim² adds to Virchow's description that the condition is more easily recognized grossly than microscopically in organs such as the liver and kidney, in which the cells normally contain some granules. He states that cloudy swelling occurs also in muscle (heart muscle, especially) and in the mammary glands.

Klebs³ and Ribbert⁴ agree with Cohnheim that cloudy swelling is more readily recognized grossly than microscopically.

Rindfleisch,⁵ Birch-Hirschfeld,⁶ Thoma,⁷ Ziegler,⁸ Albrecht,⁹ Landsteiner,¹⁰ Adami,¹¹ and von Gierke¹² all adhere closely to Virchow's description.

Benario¹³ stated that fatty granules were always to be found together with the albuminous granules in cloudy swelling.

Volhard and Fahr,¹⁴ working on the human kidney, find that the gross picture cannot be relied upon. Swelling is sometimes present and sometimes not, and the degree of cloudiness is very variable. The microscopic picture is more dependable. The epithelium of the convoluted tubules is swollen. The lumina of these tubules are decreased in size if not entirely obliterated by the swollen cells. Albuminous material is to be found in precipitated stringy masses in the lumina of some of the tubules and Bowman's capsules. The Altmann granules are enlarged and very irregular in arrangement. The rods are present in some cells.

Bell¹⁵ noted that organs will sometimes show this gross appearance of cloudy swelling when there is no increase of albuminous granules; and, conversely, that sometimes, especially in the kidney, there may be a marked increase of albuminous granules in the absence of any gross indication. He recommends that the term "cloudy swelling" be used for gross description only, and that a modifying phrase be added to indicate the microscopic picture accompanying it.

A number of observers have studied experimental cloudy swelling in animals. Favre¹⁶ in 1892 produced the condition in rabbit kidneys by removing the opposite kidney or by tying off its vein or ureter. He described an intense clouding of some of the convoluted tubules of the functioning kidney, due to the accumulation of albuminous granules within the cells.

Schilling¹⁷ produced cloudy swelling in rabbit kidneys by tying the opposite renal vein for 48 hours. At the end of this time the unoperated kidney showed a swollen grayish-yellow cortex which was easily distinguishable from the blood-red medulla. Crushed preparations showed, microscopically, opaque tubules which were filled with albuminous granules. These granules hid the nuclei almost completely. He found no fat present.

Albrecht⁹ also used rabbits for the study of experimental cloudy swelling of the kidney. He produced the condition by the following procedure. The renal artery was clamped off for two hours, after which time the clamp was removed. Within two or three hours more the clamp was again replaced and left for twenty-four hours. He obtained some fatty granules as well as albuminous granules in the tubules.

Bell¹⁵ found that there was considerable discrepancy between the gross and microscopic appearances of experimental as well as human cloudy swelling.

The term "cloudy swelling" will be used in this paper to designate a turbid swollen organ without regard to the microscopic picture that may be present.

MATERIAL AND METHODS.

Since the number and character of the granules in the renal tubules of different animals vary considerably, it was decided to use but one species. Rabbits were used exclusively. All kidney operations were made through lumbar incisions. The left kidney was operated on most frequently because it is more accessible than the right.

Compensatory changes in the kidney were studied by removing the opposite kidney or by ligature of its ureter. One form of cloudy swelling was produced by subcutaneous injections of tartaric acid. Cloudy swelling was also produced by placing sterile macerated liver in the peritoneal cavity. It was found that large subcutaneous abscesses will produce a marked increase in albuminous granules in the kidney in the course of one or two weeks. Rabbits were therefore given *pasteurella* and *proteus* infections to produce abscesses. They were killed at various stages of the infectious process. These several procedures will be explained in more detail under the separate headings.

Thin pieces of fresh kidney were fixed in Zenker-formol and 10 per cent formalin. Both these reagents preserve the granules well, but Zenker-formol gives a better cytoplasmic fixation than the formalin alone. Neither Zenker's nor Bouin's solutions could be used because the acetic acid in them destroys the granules rapidly.

Zenker-formol material was found most useful for histological study. Paraffin sections were cut at three micra and stained with iron-hematoxylin and other stains. Frozen sections were prepared from the formalin-fixed material and stained for fat. In every case crushed preparations of the fresh kidney were examined to control the fixed material and to study the effect of various reagents upon the albuminous granules.

THE NORMAL KIDNEY.

Crushed preparations of the fresh cortex usually show, microscopically, a few dark tubules and a large number of light tubules. Upon high magnification the dark tubules are seen to be filled with rather large weakly refractive granules or droplets. Upon the addition of 5 per cent NaOH or KOH these granules can be seen to disappear suddenly, like the bursting of a bubble, as the reagent comes into contact with them. This leaves the tubules light and the nuclei more clearly visible. These granules are not entirely confined to the dark tubules, for a few scattered ones are noticeable in the light tubules as well. The rods of Heidenhain can also be made out in the fresh preparations in some cases. One occasionally finds a kidney which shows no distinctly dark tubules but high magnification will always reveal occasional granules within the cells.

The granules cannot be demonstrated in tissues which have been fixed with Zenker's or Bouin's fixing fluids. Apparently the acetic acid in these reagents destroys them. Ten per cent formalin or Zenker-formol preserves the granules very satisfactorily, however.

In tissues fixed with Zenker-formol or 10 per cent formalin and stained with iron-hematoxylin, the granules are well shown (Fig. 1). They appear as darkly staining spherical bodies. They are often arranged in rows between the rods. Sometimes, however, they are found in the inner clear portion of the cells. The dark tubules are very conspicuous in stained preparations (Fig. 2).

The rods of Heidenhain can be seen in the basal portion of the cells. They are apparently independent of the granules under consideration. The inner portion of the cells is clear and has a convex inner border projecting into the lumen. The lumina of the tubules are generally narrow and stellate-shaped, due to the rounded inner borders of the cells. In some of the tubules there



Fig. 1.

Fig. 1.—Normal kidney. Zenker-formol. Iron-hematoxylin. A dark tubule is shown with parts of adjacent light tubules. X 660.

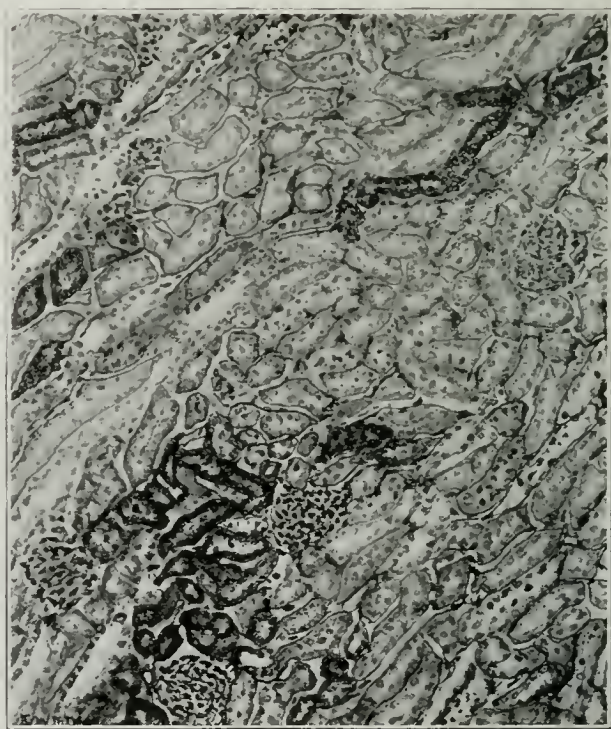


Fig. 2.

Fig. 2.—Normal kidney. Zenker-formol. Iron-hematoxylin. Showing the relative number of dark tubules. X 100.

may be no visible lumen, the inner borders of the cells meeting and entirely obliterating it.

Albrecht⁹ described the granules under consideration in the renal epithelium of rabbits and stated that he could find no previous mention of them in the literature. He thought that they were of some functional significance. Disse,¹⁸ however, in 1892 described granules in the cortical epithelium of the rabbit kidney which correspond closely to these. He did not examine them in the fresh tissue, nor did he attribute any functional importance to the granules.

Rothstein¹⁹ described these granules in the kidneys of mammals in 1890. In the fresh crushed preparations they appeared as definite refractive granules with darkly limited periphery, sometimes isolated and sometimes in groups within the cells. In fixed and stained preparations they were sometimes seen to be spherical, sometimes oval, and even sometimes spindle-shaped. He evidently considered them of functional importance.

Modragowski²⁰ mentions these granules in normal rabbit kidneys and gives them some functional significance.

Hirsch²¹ gives an accurate description of the granules and dark tubules of the normal kidney. The number of granules depends upon the functional condition of the tubules.

Bell¹⁵ also described these granules in the renal epithelium of the normal rabbit. He studied them in fresh preparations only.

No one has proven definitely that these granules have any functional significance; but they are invariably present in the normal kidney.

COMPENSATORY CHANGES IN THE KIDNEY.

Favre, Schilling, and Albrecht have all recorded the production of compensatory cloudy swelling in rabbit kidneys by the removal of the opposite kidney or by tying its vein or ureter. Favre did not describe the gross changes. He says that he obtained a clouding in some of the tubules, apparently not knowing that this is present in the normal. Schilling states that grossly the cortex was of a grayish-yellow color, easily distinguishable from the blood-red medulla. Microscopically, the tubules were opaque, due to the accumulation of albuminous granules within the cells. This author also apparently overlooked the fact that some of the tubules are dark in the normal kidney. Albrecht does not give a description of his findings.

Hirsch described an increase in the albuminous granules one week after the removal of the opposite kidney, but did not consider the gross changes worth mentioning.

Bell was unable to produce a definite gross cloudy swelling by removing the opposite kidney. From microscopic examination of fresh tissues he was not certain that there was an increase in the number of dark tubules over the normal.

EXPERIMENTS.

Experiment 1.—Rabbit; male; left kidney removed. Twenty-four hours later the animal was killed and the right kidney examined.

Gross examination. The left kidney is normal in appearance. The right kidney is swollen and congested, but no cloudiness can be seen.

Microscopic examination. No certain differences can be made out in the fresh specimens. The fixed and stained material shows a few more dark tubules in the right than in the left kidney.

Experiment 2.—Rabbit; male, weight 1,080 grams; left kidney removed. Forty-eight hours later the animal was killed and the right kidney examined.

Gross examination. Left kidney normal. The right kidney is somewhat swollen and the cut surface is moist, but there is no distinct cloudiness present.

Microscopic examination. The right kidney shows definitely more dark tubules than the left. There is also an increase in albuminous granules throughout the lighter tubules in the right kidney.

Experiment 3.—Rabbit; male; weight 2,910 grams; removed left kidney. Forty-eight hours later the animal was killed.

Gross examination. Left kidney normal. The right kidney is swollen and its cut surface is slightly pale.

Microscopic examination. The right kidney contains at least two or three times as many dark tubules as the left. The granules in the right kidney are larger than those in the left.

Experiment 4.—Rabbit; female; weight 2,000 grams. The right ureter was ligatured. One week later the animal was killed and the left kidney examined.

Gross examination. Possibly swollen. No cloudiness was detectable.

Microscopic examination. The fresh tissue shows no certain increase in the number of dark tubules. Fixed and stained material shows no increase over the normal number of dark tubules.

These experiments show that a moderate increase in the number of granules occurs, temporarily at least, after the removal of the opposite kidney. This is so slight as to be indistinguishable with certainty in the fresh specimens. It is only after a comparison of fixed and stained preparations that it can be determined. The gross changes are so slight that they cannot be recognized with certainty.

CLOUDY SWELLING PRODUCED BY TARTRATES.

Rabbits were given subcutaneous injections of racemic tartaric acid neutralized with sodium carbonate, in varying doses from .05 gm. per kilo to .15 gm. per kilo. At different times after the injection the rabbits were killed and their kidneys examined. The earlier stages were studied especially, since the later stages are of a frankly degenerative character and since many authors hold that parenchymatous nephritis passes through a preliminary stage of cloudy swelling.

Experiment 5.—Rabbit; male; weight 1,500 grams. Injected subcutaneously with 0.1 gm. racemic tartaric acid. The right kidney was removed one hour later. Two hours after the injection the animal was killed.

Right kidney: gross examination. Kidney appears normal.

Microscopic examination. Dark tubules are present in the fresh preparation. Paraffin sections show a few hydropic cells. Otherwise the appearances are normal.

Left kidney: gross examination. Cut surface is slightly pale.

Microscopic examination. Dark tubules are seen in the fresh preparations. Paraffin sections show nothing abnormal except a few hydropic cells.

Experiment 6.—Rabbit; male; weight 2,050 grams. Injected subcutaneously with 0.1 gm. racemic tartaric acid. Killed three hours later.

Gross examination. No definite changes.

Microscopic examination. Crushed preparations of fresh tissue show no dark tubules. In paraffin sections stained with iron-hematoxylin most of the convoluted tubules show a few granules. There are no dark tubules. There are a few hydropic tubules in the peripheral part of the cortex.

Experiment 7.—Rabbit; male; weight 1,800 grams. Injected subcutaneously with 0.2 gm. racemic tartaric acid. The left kidney was removed two hours later. Twenty-four hours after the injection the animal was killed.

Left kidney: gross examination. The kidney is swollen. The cut surface is succulent and a little pale.

Microscopic examination. The fresh crushed preparations show a few slightly dark tubules. These are not so prominent as in the normal. Paraffin sections show some dark tubules. The lighter tubules also contain a few granules. Hydropic tubules are noted especially in the peripheral part of the cortex.

Experiment 8.—Rabbit; female; weight 1,570 grams. Injected subcutaneously with 0.2 gm. racemic tartaric acid. The right kidney was removed three hours later. Twenty-four hours after the injection the animal was killed.

Right kidney: gross examination. The organ is slightly swollen. The cut surface is slightly paler than normal.

Microscopic examination. In the fresh preparations most of the tubules show a few granules. There are no distinctly dark tubules to be seen. Paraffin sections show extreme hydropic degeneration of the convoluted tubules. There are a few tubules which do not show this change, but none of the dark tubules are present. The normal tubules show a few scattered granules.

Left kidney: gross examination. The organ is swollen and the cut surface is distinctly cloudy.

Microscopic examination. The fresh specimen shows no dark tubules. Paraffin sections show some dilated tubules at the periphery of the cortex. These contain casts and cellular debris. There are some necrotic tubules at the periphery of the cortex. No dark tubules are present. There are no hydropic cells.

Experiment 9.—Rabbit; male; weight 1,330 grams. Injected subcutaneously with 0.2 gm. racemic tartaric acid. Killed twenty-four hours later.

Gross examination. Kidneys are swollen. The cut surfaces are succulent and a little paler than normal.

Microscopic examination. The fresh preparation shows a few dark tubules. These contain some fatty granules beside a few albuminous granules. Paraffin sections show some dilated tubules which contain casts and cellular debris. There are a number of dark tubules present. The rods are prominent in most of the tubules.

Experiment 10.—Rabbit; male; weight 2,050 grams. Injected subcutaneously with 0.3 gm. racemic tartaric acid. The right kidney was removed twenty-four hours later. Forty-eight hours after the injection the animal was killed.

Right kidney: gross examination. The organ is swollen and the cut surface is succulent and moderately cloudy.

Microscopic examination. The fresh specimen shows a large number of tubules filled with fat droplets. Paraffin sections show a large number of dilated tubules containing casts and cellular debris. There are a few of the dark tubules present. There are some necrotic tubules at the periphery of the cortex. A few convoluted tubules contain large deeply staining granules, apparently different from those in the dark tubules. Fat stain shows a large amount of fat in both the collecting and the convoluted tubules.

Left kidney: gross examination. The organ is swollen. The cut surface is only slightly pale.

Microscopic examination. There are a few fatty tubules present. Paraffin preparations show fewer dilated tubules than the right. There are some of the dark tubules present.

Experiment 11.—Rabbit; male; weight 1,970 grams. Injected subcutaneously with .3 gm. racemic tartaric acid. The left kidney was removed twenty-four hours later. Forty-eight hours after the injection the animal was killed.

Left kidney: gross examination. The organ is swollen. The cut surface is succulent and moderately cloudy.

Microscopic examination. The fresh preparation shows a large amount of fat throughout the collecting and convoluted tubules.

Right kidney: gross examination. The organ is swollen. The cut surface is slightly pale.

Microscopic examination. The fresh preparation shows a few fatty tubules. Paraffin sections show only a few dilated tubules. There are some dark tubules present. Most of the convoluted tubules contain a small amount of fat.

Experiment 12.—Rabbit; male; weight 2,050 grams. Injected subcutaneously with .3 gm. racemic tartaric acid. The left kidney was removed 7 hours later. Forty-eight hours after the injection the right kidney was removed.

Left kidney: gross examination. The organ is possibly swollen. The cut surface is succulent and pale.

Microscopic examination. The fresh preparation shows a few dark tubules. Some of the tubules contain fatty granules. Paraffin preparations show a large number of hydropic tubules. There are some dark tubules. There are dilated tubules containing casts and cellular debris.

Right kidney: gross examination. The organ is swollen. The cut surface is moist and slightly pale.

Microscopic examination. There are a few dark tubules in the fresh preparation.

Paraffin sections show no hydropic tubules. The larger number of tubules appear normal and the dark tubules are present. There are a few extremely dilated tubules which contain casts and cellular debris.

From the above experiments it will be seen that the minimum effective dose produces no gross changes in the kidneys. Larger doses, however, give a definite cloudy swelling at the end of twenty-four hours. The organ cannot be said to have a "cooked appearance" but it nevertheless is swollen and turbid. The cloudiness and swelling are in no way due to the presence of albuminous granules. The albuminous granules are never increased in number above the normal and even, at times, seem to be decreased. The opacity of the kidneys is apparently due, for the most part, to anemia, fatty droplets, and necrosis and disintegration of the cells.

CLOUDY SWELLING PRODUCED BY THE INTRODUCTION OF AUTOLYZED LIVER INTO THE PERITONEAL CAVITY.

Severe renal injury may be produced by introducing a large quantity of autolyzed liver into the body.

A rabbit's liver was removed sterilely and cut up into fine pieces. Sterile physiological saline was added to this and the tissue was allowed to autolyze in the incubator for several hours. This fluid was injected into the peritoneal cavity.

Experiment 13.—Rabbit; female; weight 1.665 grams. The urine was normal. Twenty c.c. of fluid obtained by macerating a sterile rabbit liver over night with 50 c.c. of sterile physiological saline at 37° C. was injected intraperitoneally. Four hours later the urine showed numerous hyaline casts and considerable albumin. The left kidney was removed. The animal died two days later. The urine remained about the same.

Left kidney: gross examination. The organ is swollen. The cut surface is succulent and moderately cloudy.

Microscopic examination. The fresh preparation shows no distinctly dark tubules. Some of the tubules contain a few albuminous granules, however. Paraffin preparations show a few dilated convoluted tubules. These contain sloughed off portions of cells within their lumina. The greater number of tubules appear normal. No dark tubules are present but some tubules contain a few darkly staining granules.

Right kidney: gross examination. The organ is darkened by postmortem change.

Microscopic examination. The fresh specimen shows no dark tubules although some of the tubules contain a few albuminous granules. Paraffin preparations show a large number of the tubules containing albuminous material within their lumina. There are no dark tubules but some contain a few granules.

Experiment 14.—Rabbit; male; weight 1,600 grams. Examination of the urine showed no albumin. Seventy-four gm. of sterile rabbit liver which had been macerated for five hours with 25 c.c. of sterile physiological saline was placed in the peritoneal cavity. Eighteen hours later the left kidney was removed. The urine was cloudy with granular casts and contained a large amount of albumin. Eight days later the animal died. During this time the urine remained about the same.

Left kidney: gross examination. The organ is swollen. The cut surface is extremely cloudy and succulent.

Microscopic examination. The fresh preparation shows no dark tubules. Paraffin sections show a pronounced parenchymatous nephritis but no dark tubules.

Right kidney: gross examination. The organ is swollen. The cut surface is succulent and moderately pale.

Microscopic examination. The fresh specimen shows no dark tubules. There are a large number of fatty granules present. Paraffin sections show considerable interstitial edema. There are a few tubules which contain some albuminous granules. Some of the tubules are dilated and contain casts and cellular debris.

No increase in the number of albuminous granules occurred in these experiments. Instead, the granules entirely disappeared and a very pronounced parenchymatous nephritis resulted. In spite of the disappearance of the granules an extreme degree of cloudy swelling occurred. This must be taken as further proof that the swelling and clouding of the kidney and the accumulation of albuminous granules within the cells may be the results of distinctly different pathological processes.

(Whether the changes in the kidney were due to autolyzed proteins or to the bile salts present was not determined.)

CLOUDY SWELLING PRODUCED BY INFECTIONS.

In this group of experiments the conditions simulate some of the human diseases in which a cloudy swelling may occur. Bell mentioned the occurrence of an albuminous degeneration (cloudy swelling) in rabbit kidneys in a case of infection.

Cultures taken from a large pasteurella abscess were used to inoculate other rabbits. The animals were weighed each day and the progress of the infection was measured by the loss in weight. By killing the animals at different stages of the infectious process different degrees of cloudy swelling were obtained.

Most of the cases studied were pasteurella infections, since these organisms infect rabbits very readily. Some animals with proteus abscesses and one with a streptococcus infection were studied. The renal changes were similar in all three types of infections.

Experiment 15.—Rabbit; male; weight 1,040 grams. Inoculated subcutaneously with pasteurella. An abscess developed. Thirteen days later the animal was killed. Weight 890 grams.

Kidneys: gross examination. There are no marked changes from the normal.

Microscopic examination. The fresh specimen shows a large number of dark tubules. There are no fatty granules. Paraffin sections show considerably less than half the tubules dark (Fig. 3). These are crowded with granules which tend, in some tubules, to be arranged in rows in the basal part. The rods are present between the granules and are very prominent in the clear tubules. The cells of the tubules are swollen so that no lumina are visible. Frozen sections stained with sudan iii show that no fat is present.

Experiment 16.—Rabbit; male; weight 1,900 grams. The animal was inoculated subcutaneously with pasteurella. A large abscess developed. Fourteen days later the animal died. Weight 1,300 grams.

Kidneys: gross examination. The organs show some postmortem discoloration. They are not swollen.

Microscopic examination. The fresh specimen shows numerous dark tubules filled with albuminous granules. No fatty granules are present. Paraffin sections show numerous dark tubules filled with deeply staining granules. These are confined to the basal part of the cells in only a few tubules. The rods are not present. No fat can be demonstrated in frozen sections.

Experiment 17.—Rabbit; male; weight 2,010 grams. Phenolsulphonephthalein test, 75 per cent for the first two hours. The urine was normal. Inoculated subcutaneously with pasteurella. An abscess developed. Ten days later phenolsulphonephthalein test was 75 per cent. Two days later an examination of the urine showed a faint trace of albumin and a few hyaline casts. Thirteen days after inoculation the animal died. Weight 1,360 grams.

Kidneys: gross examination. The organs show some postmortem discoloration. They are not swollen.

Microscopic examination. The fresh specimen shows numerous dark tubules contain-

ing albuminous granules. Paraffin sections show the cells of slightly less than half the tubules filled with albuminous granules. A few of the tubules have wide lumina and fragmented cells. There is often albuminous material within the lumina of the tubules. The rods are not present.

Experiment 18.—Rabbit; male; weight 1,470 grams. The rabbit had had a spontaneous abscess on the jaw which had been noticed for about a month. Nine days after I received the rabbit it was killed. Weight 1,170 grams.

Kidneys: gross examination. The organs were not swollen. The cut surfaces are moderately cloudy.

Microscopic examination. The fresh preparation shows a large number of dark tubules which are filled with albuminous granules. Paraffin preparations show the cells of slightly more than half the tubules filled with deeply stained granules. The rods of Heidenhain are prominent in places between the granules. No fat is present.

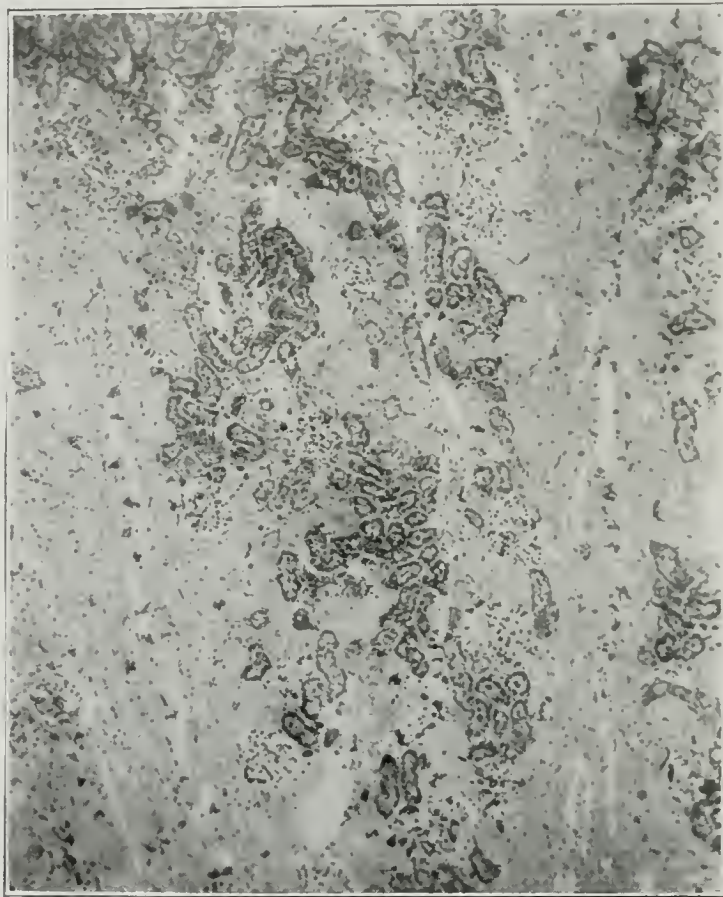


Fig. 3.—Exp. 15. Kidney from a case of chronic infection. Zenker-formol. Iron-hematoxylin. There is a considerable increase in the number of dark tubules. Photomicrograph. X 80.

Experiment 19.—Rabbit; male; weight 2,000 grams. The left kidney was removed. One week later the rabbit was inoculated with *B. proteus*. A diffuse infection resulted. Eight days after the inoculation the animal died.

Right kidney: gross examination. The organ is markedly swollen. The cut surface is succulent and slightly pale.

Microscopic examination. The fresh preparation shows numerous dark tubules filled with albuminous granules. Some of these granules are very large. Paraffin sections show the cells of slightly more than one-half of the tubules filled with deeply staining granules. The rods are not present. (The rabbit had been dead for some time when it was found.) The lumina of most of the tubules are obliterated by the swollen cells. No fat can be demonstrated.

Experiment 20.—Rabbit; female; weight 1,850 grams. Inoculated subcutaneously with pasteurilla. An extremely large abscess developed. Nine days later the left kidney was removed. Weight 1,330 grams. The next day the animal was killed.

Left kidney: gross examination. The organ is swollen. There is no noticeable cloudiness.

Microscopic examination. The fresh specimen shows numerous dark tubules which are filled with albuminous granules. These granules are very irregular in size. Paraffin preparations show a little less than one-half the tubules dark. These are crowded with darkly staining granules. The granules are very large in certain tubules. The rods of Heidenhain are present between the granules in the basal portion of the cells. Sections stained for fat show a little present in a few of the convoluted and collecting tubules.

Right kidney: gross examination. The organ is swollen. The cut surface is slightly cloudy.

Microscopic examination. The microscopic examination shows a few more dark tubules than in the left kidney. Otherwise they are the same.

Experiment 21.—Rabbit; male; weight 1,520 grams. Phenolsulphonephthalein test, 80 per cent. Inoculated subcutaneously with *B. proteus*. Six days later, phthalein test, 75 per cent. Nine days after inoculation, phthalein test, 50 per cent. Killed ninth day after inoculation. Weight, 1,200 grams.

Kidney: gross examination. The organ is swollen. The cut surface is succulent and cloudy.

Microscopic examination. Fresh preparations show a large number of dark tubules present which contain albuminous granules. Paraffin preparations show over one-half of the tubules filled with darkly staining granules. The rods are well preserved. Fat stains show only a very few fatty granules in some of the tubules.

Experiment 22.—Rabbit; male; weight 1,610 grams. Phenolsulphonephthalein test, 70 per cent. The rabbit was inoculated subcutaneously with *B. proteus*. A diffuse infection resulted. Three days later the phthalein test was the same. Five days after the inoculation the animal died. Weight 1,090 grams.

Kidneys: gross examination. No swelling is present. The cut surfaces are succulent but not cloudy.

Microscopic examination. The fresh preparation shows a large number of moderately dark tubules. Paraffin preparations show that about half of the tubules are dark. The number of granules is less than in pronounced cases. Considerable fat was demonstrated in some of the convoluted tubules and in the collecting tubules.

Experiment 23.—Rabbit; male; weight 1,910 grams. Urine examination showed nothing abnormal. Phenolsulphonephthalein test, 80 per cent. Inoculated subcutaneously with *B. proteus*. A large abscess developed. Nine days later, phthalein test, 70 per cent. The urine showed a faint trace of albumin but no casts. Eleven days after inoculation the left kidney was removed. Weight 1,660 grams. Death on the twelfth day.

Left kidney: gross examination. The organ is swollen. The cut surface is succulent and markedly cloudy.

Microscopic examination. The fresh specimen shows an extremely large number of dark tubules filled with albuminous granules. Paraffin preparations show more than half of the tubules dark, due to the presence of deeply stained granules within the cytoplasm of the cells. The rods of Heidenhain are present in the basal part of the cells between the granules. The cytoplasm of the cells in all the tubules is intact. No fat is present.

Experiment 24.—Rabbit; male; weight 1,555 grams. The left kidney was removed. An abscess developed at the site of the operation. Killed 18 days after nephrectomy.

Right kidney. The organ is swollen. The cut surface is succulent and extremely cloudy.

Microscopic examination. Fresh preparations show extremely numerous dark tubules. Paraffin sections show most of the tubules dark, due to the presence of deeply stained granules. Sections stained for fat show none present.

Experiment 25.—Rabbit; female; weight 2,210 grams. Inoculated with *B. proteus*. A large abscess developed. Twelve days later the animal died. Weight 1,775 grams.

Kidneys: gross examination. The organs are markedly swollen. The cut surface is succulent and distinctly cloudy.

Microscopic examination. The fresh specimen shows a large number of dark tubules which are filled with albuminous granules. Paraffin sections show the cells of a majority of the tubules filled with albuminous granules. The rods of Heidenhain are not present. (The kidneys were not obtained until some time postmortem.) The cytoplasm of the cells appears intact in all the tubules.

Experiment 26.—Rabbit; female; weight 1,730 grams. Inoculated subcutaneously with

pasteurella. An abscess resulted. Twenty-three days later the animal was killed. Weight 1,105 grams.

Kidney: gross examination. The organ is not swollen. The cut surface is moist and moderately cloudy.

Microscopic examination. There are numerous dark tubules filled with albuminous granules. Paraffin sections show the greater number of the convoluted tubules dark and filled with granules. These granules tend to be confined to the basal portion of the cells. The rods are prominent between the granules. The cells of most of the tubules are swollen so that no lumen is visible. There are a few tubules, however, which have distinct lumina and these often contain albuminous material. No fat could be demonstrated in frozen sections.

Experiment 27.—Rabbit; male; weight 1,850 grams. The animal was recovering from a pasteurella abscess. It was inoculated with pasteurella. Fourteen days later it was killed. Weight 1,565 grams.

Kidney: gross examination. The organ is moderately swollen. The cut surface is slightly cloudy and succulent.

Microscopic examination. The fresh preparation shows numerous dark tubules which are filled with albuminous granules. Paraffin sections show most of the tubules dark, due to the presence of darkly staining granules within the cells. In a few tubules they are arranged in rows between the rods of Heidenhain. The rods of Heidenhain are present in places between the granules and are very prominent in the less granular tubules. There are some dilated tubules which contain casts and albuminous material. No fat could be demonstrated.

Experiment 28.—Rabbit; male; weight 1,945 grams. The urine was normal. Phenol-sulphonephthalein, 81 per cent. Inoculated subcutaneously with *B. proteus*. An abscess developed. Nine days later the urine was normal and the phenolsulphonephthalein test was about the same. Eleven days after inoculation the animal was killed.

Kidneys: gross examination. The organs are markedly swollen. The cut surfaces are definitely cloudy and succulent.

Microscopic examination. The fresh preparation shows a large number of dark tubules. Paraffin sections show that almost all the tubules are dark. The granules vary considerably in size. The rods are well preserved and prominent.

Experiment 29.—Rabbit; male. Inoculated with pasteurella. A large subcutaneous abscess developed. Three weeks after inoculation the animal was killed.

Kidney: gross examination. No swelling is present. The cut surface is distinctly cloudy.

Microscopic examination. The fresh preparation shows numerous dark tubules with albuminous granules. Paraffin preparations show almost all the convoluted tubules dark, due to the presence of numerous darkly staining granules within the cells. These granules vary in size. Some are half the size of the nucleus. The rods show in the basal part of the tubules between the granules. The cytoplasm of the cells of all the tubules is intact. No fat is present.

Experiment 30.—Rabbit; male; weight 1,560 grams. Phenolsulphonephthalein test, 75 per cent. Inoculated subcutaneously with *B. proteus*. An abscess developed. Thirteen days later phthalein test was 85 per cent. The urine showed a trace of albumin but no casts. The animal died shortly after the phthalein test was made.

Kidneys: gross examination. The organs are not swollen. The cut surfaces are slightly pale. The inner part of the medulla is extremely light colored.

Microscopic examination. The fresh specimen shows numerous dark tubules. Paraffin sections show that almost every convoluted tubule is dark. The rods are distinctly visible in most of the tubules. No fat is present.

Experiment 31.—Rabbit; male; weight 2,140 grams. Inoculated subcutaneously with pasteurella. A large abscess resulted. Twelve days later the animal died. Weight 1,365 grams.

Kidneys: gross examination. There is no swelling present. The cut surface is moist and definitely cloudy.

Microscopic examination. The fresh specimen shows numerous dark tubules filled with albuminous granules. Paraffin sections show almost all the tubules dark, due to the presence of darkly staining granules. In but few tubules are they confined to the basal

part of the cells. The rods are present in the basal part of the cells between the granules. The cells of the tubules are swollen so as to obliterate the lumina.

Zenker fixation. Paraffin preparations show no dark tubules. The granules are absent with the exception of a few scattered ones in some of the tubules. These do not stain intensely with iron-hematoxylin.

Experiment 32.—Rabbit; male; weight 2,260 grams. The animal was inoculated subcutaneously with pasteurella. A large abscess resulted. Twelve days later the left kidney was removed. Weight 1,690 grams. Three weeks after the inoculation the animal was killed. Weight 1,325 grams.

Left kidney: gross examination. The organ is swollen. The cut surface is moist and slightly cloudy.

Microscopic examination. The fresh preparation shows numerous dark tubules filled with albuminous granules. In some of the cells the granules are as large as the nucleus. There are a few granules which do not burst upon the addition of alkali but dissolve

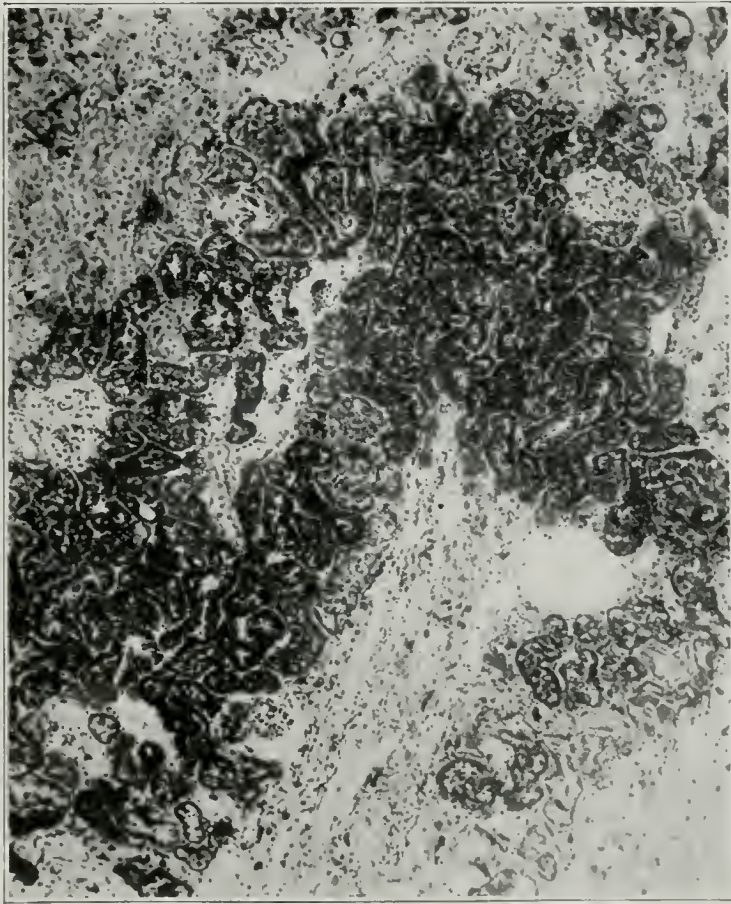


Fig. 4.—Exp. 32. Kidney from a case of chronic infection. Zenker-formol. Iron-hematoxylin. There is a very marked increase in the number of dark tubules. Photomicrograph. X 80.

gradually. These are less refractive than the other granules. Paraffin preparations show the greater number of tubules to be extremely dark, due to the accumulation of darkly staining granules within the cells (Fig. 4). In some tubules these granules are immense, being as large as the nucleus (Figs. 5 and 6). Most of the granules are spherical, but some are oval and others spindle-shaped. The rods are distinctly visible in places between the granules. The cells of the tubules are swollen so that the lumina are almost all obliterated. No fat is present.

Right kidney: gross examination. The organ is swollen. The cut surface is extremely cloudy and succulent.

Microscopic examination. There are more dark tubules than in the left kidney and the granules are larger. Otherwise the kidneys are the same. There is no fat present.

Zenker fixation (right kidney). Paraffin sections. The granules are absent except in relatively few tubules. There are numerous light spaces in the cytoplasm of the cells. These are probably the spaces from which the granules have been dissolved out.

Experiments 33-50.—In a large number of experiments the rabbits died within one to

four days after the inoculation. The pictures presented by the kidneys in these cases were almost absolutely uniform and may all be described together. Grossly, the kidney is swollen. The cut surface is succulent but not cloudy. The inner part of the medulla is often extremely light colored. Microscopically, the fresh tissue shows no dark tubules. There is often fat present in the form of small droplets. Paraffin preparations show no dark tubules. There are usually a few granules in some of the tubules. There are usually some tubules in which the cells are disintegrated. The lumina of these tubules are wide and contain albuminous material or casts. The rods are absent or if present at all may be made out in only a part of the tubules. There are usually a number of small fat droplets in the collecting tubules and in many of the convoluted tubules near the medulla.

It will be seen from the foregoing that the experiments on infection may be divided into two groups: (1) a chronic or subacute type in which the rab-

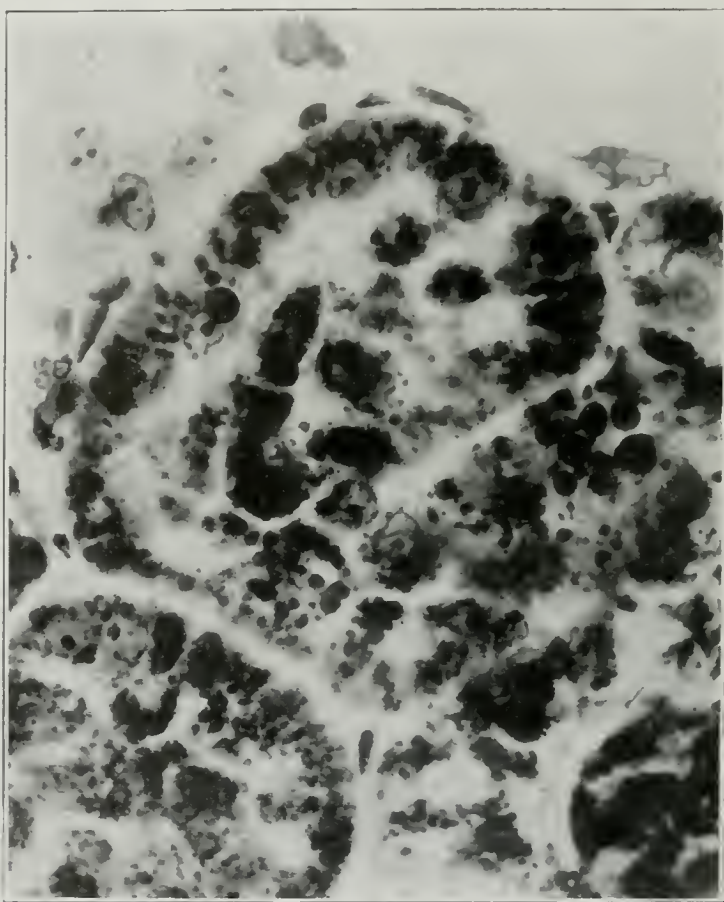


Fig. 5.—Exp. 32. Small area from Fig. 4. The granules are very large and occupy the greater part of the cells. Photomicrograph. X 500.

bits lived more than a week; (2) an acute type in which the animals died within the first few days.

The characteristic picture found in kidneys of the first group of animals (Exps. 15 to 32) is as follows:

Gross examination. The gross picture is variable. In some cases the cortex is distinctly cloudy and swollen, but in other cases these features are barely noticeable. The medulla rarely shows any change. The cut surface is moist.

Microscopic examination (fresh). The fresh crushed specimen shows, under low power, a large number of dark tubules. These are seen upon high magnification to be filled with weakly refractive, definitely circumscribed granules, which almost conceal the nuclei. Upon addition of alkali these granules disappear like the bursting of a bubble, leaving the tubules light and the nuclei

clearly visible. These granules are also present in the light tubules, but they are fewer and scattered. In the more pronounced cases almost every convoluted tubule may be dark. In these instances some tubules contain immense granules, almost as large as the nucleus. These also burst upon the addition of alkali. No fatty granules are present.

In severe cases there appears another type of granule. This is less refractive than the former type and dissolves gradually in alkali.

Fixed and stained preparations. Hematoxylin and eosin. The cells of the convoluted tubules appear swollen, their inner borders projecting into and often obliterating the lumina. The cytoplasm stains well and, with low power,

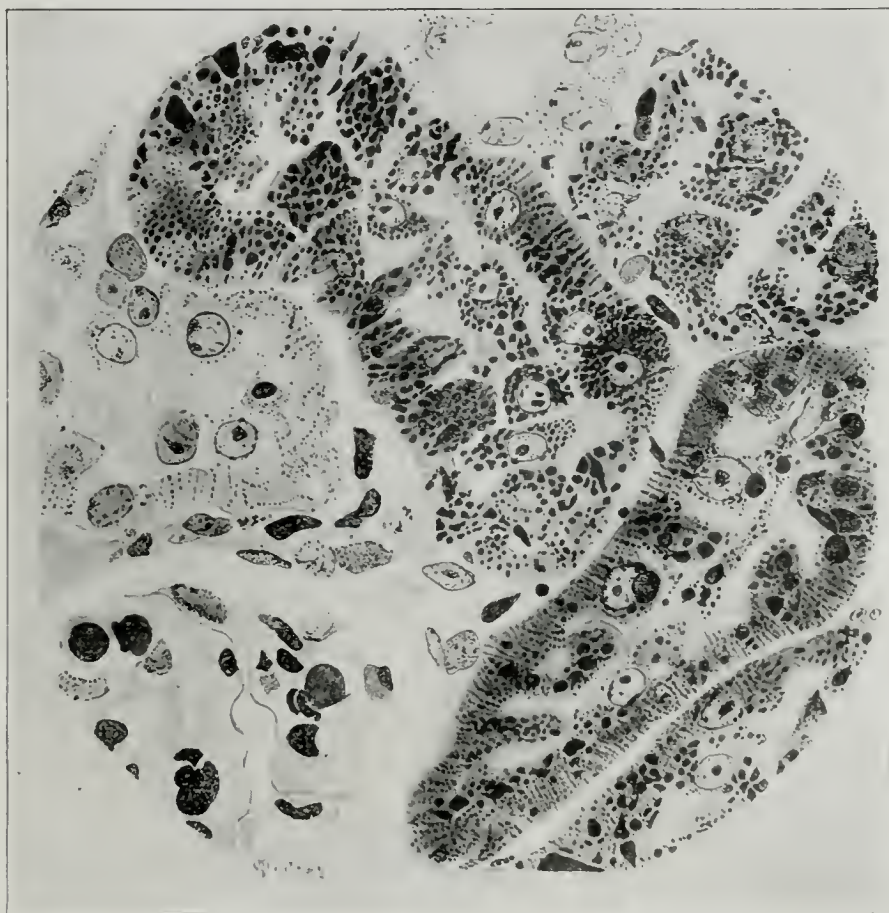


Fig. 6.—Exp. 32. Drawing of small area from Fig. 4, showing details of structure. X 660.

seems to be perfectly homogeneous. Upon examination with oil immersion, however, the granules can be seen as bright red staining bodies.

Iron-hematoxylin. The cells of the greater number of convoluted tubules are seen to be filled with intensely staining granules. These are, as a rule, more numerous in the basal portion where they often show a tendency to be arranged in rows. In the inner portion of the cells they are irregularly scattered through the cytoplasm. In some tubules the granules are distributed uniformly through the whole cell. As described in the fresh specimen, the size of the granules varies. In the more pronounced cases they are very irregular in size, some being as large as the nucleus. Most of the granules appear spherical, but there are also oval and spindle-shaped ones in some of the tubules. The rods of Heidenhain may be seen in places between the masses of granules and are very prominent in the less granular tubules. There is no fat present.

The characteristic picture of kidneys of the second group of animals (Exps. 35-50) is as follows:

Gross examination. The gross, as a rule, shows no change, but sometimes the organ is succulent and swollen. In some cases the inner part of the medulla is extremely pale.

Microscopic examination. In fresh preparations there are no dark tubules. There are generally a few albuminous granules, however, scattered through the tubules. There are often scattered albuminous granules which do not dissolve upon the addition of acid or alkali. These are often particularly numerous in the glomeruli.

Fixed and stained preparations. Hematoxylin and eosin. There is always a varying number of tubules with wide lumina containing albuminous material and sloughed off portions of cells. The inner borders of the cells in many of the tubules contain vacuoles. These often give a ragged appearance to the cells.

In fixed material stained with iron-hematoxylin, the dark tubules are found to be entirely absent. A varying number of tubules contain small sparsely scattered granules which do not often show any tendency to be arranged in rows. The rods are absent as a rule. In some of the kidneys they are present in a few of the tubules, but absent in the greater number. In these acute cases there are often darkly staining bodies in the glomeruli. There is considerable fat in the form of fine granules in the collecting tubules and in the convoluted tubules near the medulla.

The most striking difference between the kidneys of acute and those of chronic infections is the almost complete absence of albuminous granules in the former and their presence in enormous numbers in the latter.

In the acute cases the renal tubules always show much more marked signs of injury than they do in the chronic cases. In the chronic group the phthalein test remains usually about normal and the cell structure is well preserved. These facts suggest that the marked increase of albuminous granules is not a degenerative change.

The number of dark tubules is greatest in those cases where there has been an extreme but very gradual loss of weight (Exps. 29-32). The albuminous granules in the dark tubules in the chronic cases are larger and more numerous than those in the dark tubules of a normal kidney. In some cases they attain an enormous size, sometimes being as large as the nucleus. The large granules are probably formed by fusion of smaller granules, since the granules are fewer in cells containing the large types.

The albuminous granules in the chronic cases closely resemble the granules of the dark tubules of the normal kidney. They burst when sodium hydroxid is applied, and they disappear in acetic acid solutions. They are fixed and stained by the same procedures. They are entirely different from the hyaline granules often seen in human nephritic kidneys. They are evidently thin-walled vesicles filled with fluid and not solid bodies.

In very severe infections one often sees granules that are less refractive than those described above. These granules disappear gradually in sodium hydroxid. They do not burst in this solution.

There is no fat present in the chronic cases. There is no evidence that

this increase of albuminous granules is in any way related to the formation of fat.

Fat droplets appear in the acute cases. It seems that toxic substances which cause the formation of fat droplets at the same time destroy the albuminous granules.

The gross changes in the kidneys of the chronic cases usually vary in degree, depending upon the number of albuminous granules present. Sometimes, however, there are many more granules present than is suggested by the gross picture. The kidneys are sometimes very opaque, but a typical "cooked appearance" was never obtained.

EFFECT OF ACUTE INFECTIONS UPON THE KIDNEYS OF CHRONIC CASES.

It has already been shown (Exps. 33-50) that acute infections cause the disappearance of the albuminous granules of normal kidneys. Exps. 51 and 52 were made to determine the effect of acute infections upon the kidneys of chronic cases which contain enormous numbers of albuminous granules.

Experiment 51.—Rabbit; male; weight 2,395 grams. The animal was inoculated with proteus. Four days later the left kidney was removed. Weight 2,175 grams. The animal was then reinoculated with a large dose of proteus intended to be rapidly fatal. Death the next day. Weight 2,080 grams.

Left kidney: gross examination. The organ is swollen. There is no cloudiness present.

Microscopic examination. Dark tubules are present in the fresh preparation. Paraffin sections show about half of the tubules moderately dark. These tubules contain fewer dark granules than the dark tubules of more severe infections. The rods are well preserved. The cytoplasm of the cells is intact in all of the tubules.

Right kidney: gross examination. The organ is swollen. The cut surface is succulent but not cloudy. The inner part of the medulla, however, is extremely pale.

Microscopic examination. The fresh preparation shows no dark tubules although there are a few albuminous granules in some of the tubules. Paraffin preparations show no dark tubules. The cytoplasm of the cells of many of the tubules is fragmented. The lumina of these tubules are wide and often contain albuminous material. The rods are well preserved in only a few tubules.

Experiment 52.—Rabbit; male; weight 1,890 grams. Phenolsulphonephthalein test, 68 per cent. The animal was inoculated subcutaneously with *B. proteus*. Thirteen days later the phthalein test was 70 per cent. Weight 1,150 grams. The animal was then given a very large dose of *B. proteus*. Death the next day.

Kidneys: gross examination. The kidneys are swollen. The cut surfaces are succulent and moderately cloudy.

Microscopic examination. There are no dark tubules in the fresh preparation. Paraffin sections show no dark tubules. There are a few scattered darkly staining granules in some of the tubules, however. The rods of Heidenhain are present in most of the tubules. A few of the tubules are dilated and contain casts and albuminous material within their lumina. The cells of a few tubules are fragmented on their inner borders. Sections stained for fat show a considerable quantity present in the convoluted and collecting tubules in the form of fine granules.

ETIOLOGY AND ULTIMATE SIGNIFICANCE OF CLOUDY SWELLING.

Various interpretations of cloudy swelling are to be found in the literature. The ideas may be grouped as follows:

1. Excessive functional activity. This was the belief of Virchow. He regarded the granules as nutritive material taken into the cells but not assimilated. Birch-Hirschfeld recognized one kind of cloudy swelling of this type.

and another in which the granules represent surplus waste material. Albrecht and Landsteiner interpret some cases of cloudy swelling (compensatory) as due to increased physiological activity. Adami believes that the granules are due to excessive and prolonged stimulation of the cells.

2. Coagulation or precipitation theory. Cohnheim, Rindfleisch and Klebs believed that the granules are formed from the coagulation of fluid albumins in the cells. Klebs and Fischer²² explained the coagulation as due to acid formation in the cytoplasm.

3. Degeneration theory. Many authors regard cloudy swelling as essentially a degenerative process. It is explained as due to decreased oxidation (Benario), to poisonous substances (Ribbert, Schilling), and to decreased nutrition (Thoma). Others who subscribe to this view are Birch-Hirschfeld, Albrecht (one type of cloudy swelling), Volhard and Fahr. Some observers emphasize the intimate relation between cloudy swelling and fatty metamorphosis (Benario, von Recklinghausen, Ziegler, Birch-Hirschfeld, Ribbert, Rindfleisch, Wells,²⁴ Thoma, and Adami). Many believe that cloudy swelling is a process that may readily pass into definite cell degeneration and necrosis (Virchow, Cohnheim, Ziegler, Ribbert, Rindfleisch, Thoma, Adami, Volhard and Fahr).

4. Emulsification theory. Albrecht believes that one form of cloudy swelling represents an emulsification of the cytoplasm, due to imbibition of fluid and separation of lipoidal substances. Von Gierke and Anitschkow²⁵ accept this interpretation in a modified form.

Albrecht recognized three fundamental types of cloudy swelling: emulsification, excessive function, and disintegration. Some other observers also believe that there is more than one fundamental process concerned.

Nearly all the authors mentioned above have worked exclusively with human cloudy swelling. It is easily possible that some of the processes involved here are different from those concerned in the rabbit kidney.

My observations indicate that the cloudy swelling occurring in tartrate nephritis is due to edema, anemia, fat, tissue disintegration, etc., and is not associated at all with albuminous granules.

But the cloudy swelling of the kidney following chronic infections is evidently due in part to a marked increase of albuminous granules in the cells. This type of cloudy swelling is probably not a degenerative process, since the phthalein test nearly always shows normal functional activity (Exps. 17, 23, 28, 30). It seems to be a physiological response to an increased quantity of protein waste products in the blood.

DISCUSSION.

Much of the confusion in the diagnosis of cloudy swelling is due to lack of agreement among pathologists as to what constitutes the gross picture. If we make the diagnosis only when the organ looks "cooked," then we shall almost invariably find this gross picture associated with a marked increase of albuminous granules in the cells. If we take the position that this classical picture and this alone constitutes cloudy swelling, then the condition becomes very rare. All the milder degrees of this same process would be excluded, since the typical "cooked" appearance occurs only when the cells are crowded with al-

buminous granules. This definition of cloudy swelling would also exclude all the experimental lesions described in this paper, since none of them had the typical "cooked" appearance.

The average pathologist often makes a diagnosis of cloudy swelling in an organ that is merely turbid and swollen, in the absence of the typical "cooked" appearance, and without a microscopic examination of the fresh tissue. If the term is used in this sense then cloudy swelling becomes one of the most frequent of autopsy findings. Cloudiness and swelling in an organ may be due to edema, anemia, fat, albuminous granules, tissue disintegration, etc. This use of the term obviously makes it include several distinct pathologic phenomena.

In my experiments the kidneys of animals injected with tartrates and autolyzed liver tissue were often swollen and cloudy. These changes in the gross appearances were apparently due to edema, anemia, tissue disintegration, etc.

The kidneys from the cases of chronic infections were also often cloudy and swollen, but here the gross changes were due mainly to an enormous increase of albuminous granules.

In my opinion the term cloudy swelling should be dropped out of pathologic literature. The classical cloudy swelling is merely an intense accumulation of albuminous granules in the cells and is more clearly described as such. If the term is to be used to describe a turbid swollen organ, it becomes worse than useless, since it groups together several unrelated pathologic phenomena.

There would be less confusion if the term cloudy swelling were discarded entirely and the several phenomena which produce this appearance were considered separately. Thus, instead of studying cloudy swelling, we should study the increase of albuminous granules, edema, fatty metamorphosis, emulsification of the cytoplasm, etc.

SUMMARY.

The normal rabbit kidney always contains, in the convoluted tubules, coarse albuminous granules. Usually these granules are so numerous in a few tubules that these appear dark in the fresh tissue. The granules are apparently thin-walled vesicles filled with fluid. They are best fixed by solutions containing formalin. They are not fixed in solutions containing acetic acid.

When one kidney is removed the dark tubules are increased in the opposite kidney during the first twenty-four or forty-eight hours, but the increase of albuminous granules is not sufficient to cause any definite change in the gross appearance of the kidney.

Subcutaneous injections of tartrates produce a swollen, cloudy kidney, but there is no increase of albuminous granules. The cloudiness and swelling are apparently due to edema, anemia, tissue disintegration, etc.

Intraperitoneal injections of autolyzed liver tissue produce a markedly cloudy and swollen kidney. The albuminous granules disappear entirely. The gross changes are apparently due to the same factors concerned in the tartrate experiments.

Chronic suppurative processes attended with marked emaciation cause an enormous increase of albuminous granules in the kidneys. These granules are

often larger than the normal and irregular in shape, but they seem to have the same chemical composition.

Acute toxemias cause a rapid disappearance of the normal albuminous granules.

An acute toxemia superimposed upon a chronic suppurative process causes a disappearance of the albuminous granules.

Kidneys which show an enormous increase of albuminous granules usually give a normal phthalein output, and the cells are usually intact. This form of cloudy swelling is therefore probably not a degenerative change, but a physiological response to an increase of protein waste products in the blood.

There is no relation between the formation of albuminous granules and fatty metamorphosis.

It is suggested that the term cloudy swelling be discarded and that the several processes producing this appearance be considered separately.

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PROGRESSIVE LENTICULAR DEGENERATION*

BY FREDERIC J. FARNELL, M.D., PROVIDENCE, R. I., AND ARTHUR M. HARRINGTON, M.D., HOWARD, R. I.

IN 1912 Kinnier Wilson¹ described in detail lenticular disease and reported several cases with necropsies. During the last three years several observers have expressed their views as to the exact function of the nuclei of the corpus striatum. Mingazzi² has referred to its function in relation to the motor disorders and sensory accompaniments. He also states that should four-fifths of the left nucleus be involved that dysarthria might occur. Stocker,³ in 1913, reported as a case of lenticular disease, one in which the external ocular muscles were involved in addition to the cardinal symptoms as described by Wilson, still the tremor was only slight and inconstant. Autopsy showed, however, a lobular atrophy of the liver and softening of both corpus striatum. He raises the question of differential diagnosis between lenticular degeneration and multiple sclerosis, diffuse sclerosis, senile and juvenile paralysis agitans. H. Oppenheim,⁴ in 1914, described three cases which he classed as cases of pseudosclerosis. All his cases had a constant general body tremor, disturbances in speech and emotional instability. His feeling was that they were closely allied to Wilson's progressive lenticular degeneration, but he did not refer to the fact that in cases heretofore described as pseudosclerosis there was no disturbance in the liver whereas in Wilson's cases the liver showed, invariably, a toxic cirrhosis. A case report, described by Mills⁵ late in 1914 revealed at necropsy an atrophy of a large part of both caudate nuclei and a symmetrical sclerosis of the anterior portion of each lenticular nucleus. In his discussion he refers especially to the symptoms referable to the caudate nucleus such as hypertonicity and paresis of the affective motor apparatus, painful emotional expressions and various other symptoms probably in relation to the cortico-autonomic nervous system. In addition he relates its possible effect upon the vasomotor system, causing disturbances in temperature, pulse and glandular activity. In his case, as in the one here to be reported, there was also infringement upon the internal capsule. There is little doubt, however, but that more of the symptoms were due to extra-pyramidal lesions and should contribute a few facts as to the pathogenesis of extra-pyramidal disorders. Cadwalader⁶ has reported a case with autopsy at which time a bilateral lesion of the lenticular nuclei and a cirrhotic liver were found.

It is because of the great interest that is being demonstrated in relation to the functions of the lenticular nucleus and the symptoms produced by its disease that it was deemed opportune to report this interesting case of lenticular disease with the autopsy findings.

CASE.—H. H., female, age 19, no occupation, single.

Family History.—It was considered negative for two generations. The father died of pulmonary tuberculosis and the mother from heart disease. One

*From the Clinico-pathological Department, Rhode Island State Hospital for Insane. Presented before the New York Neurological Society, January 4, 1916.

brother and two sisters are alive and well. No history of nervous or mental disease as far as could be ascertained.

Personal History.—The patient was the fourth in a family of six children. She had measles as a child. She attended school from five until fourteen, reaching the sixth grade. Her reason for not reaching a higher grade was that she had to remain at home to take care of her mother. During the summer of 1912 she was employed in a rubber factory, also in a stocking factory for two weeks. She left the first place because she was nervous. She was discharged from the second position because of pediculæ capitis. The patient never menstruated. She had no serious illness until the present.

Present Condition.—Her troubles appear to date back four years when she began to have epileptoid seizures, the description of which it has been hard to obtain. The patient called them convulsions. She had three at or about the time of the death of her mother in 1912. Since that time she has been "nervous," suffering from a coarse tremor of the hands and legs which caused her great difficulty in coordinating. During the month of May, 1913, she became so incapacitated that she was admitted to the Rhode Island General Hospital.

*Abstract from the Rhode Island General Hospital.**—The patient was well nourished. She had no disturbance in her internal viscera as far as could be ascertained. She had no pupillary disorder. The superficial abdominal reflexes were absent. The knee-jerks were exaggerated unequally, the right greater than the left. There was a suggestion of a Babinski sign on the left side. The gait was somewhat spastic. There was no disturbance in sensation. The extremities evinced evidence of a poor circulatory condition. The most characteristic feature of the disorder as observed in the hospital was the coarse incoordinate tremor of both arms, her refusal to talk and her refusal to eat at times. While in the hospital, on July 25, she had her first epileptoid seizure, resembling true epilepsy but thought to be hysterical. She had several of these attacks while under observation but they were finally thought not to be especially characteristic. During her stay, from May to October, her condition became progressively worse with the addition of emotional instability and aphonia. Dementia præcox, hysteria and multiple sclerosis were considered. She was discharged October 14 to go to the State Almshouse.

*Abstract for the State Almshouse.**—The patient was described as being excited, hilarious and noisy. She made attempts to escape. She was filthy, spat at people; she was violent and kicked the nurses. She was resentful. Observations indicated that the question of epilepsy had not been satisfactorily settled; she did not have any seizures and with the exception of fits of temper and destructiveness as well as an uncontrollable rage, there was nothing to suggest epilepsy. Most of the time she was in a state of great emotional and mental excitement with psychomotor unrest. She was resistive at times and again quiet. She sang, hummed, cried and swore, using much vulgarity colored with sexual thoughts. Observations pointed to the fact that she had had some spinal cord disorder somewhat similar to anterior poliomyelitis. She was discharged Feb-

*We wish to express our appreciation to Dr. John M. Peters of the Rhode Island Hospital and to Dr. Henry A. Jones of the State Almshouse for their kindness in furnishing us with abstracts.

ruary 5, 1914, as a case of hysterical insanity and transferred to the State Hospital for the Insane.

At the *State Hospital for the Insane* her physical condition was characterized by choreiform movements of the extremities, unsteadiness in gait and a profuse, loquacious stream of thought without any defects in memory, disturbance of thought or appearance of trends. She was excited, talkative, fearful and destructive. There were no delusions nor hallucinations. She was perfectly well oriented for time, place and person. Consciousness showed no disturbance and there was no evidence of mental confusion. Her attention was readily gained but poorly held owing to her distractability. Her trend of thought was spontaneous and coherent with occasional periods of distractability. Intellectually she appeared to be somewhat inferior for a girl of her age.

She was placed in the continuous bath several times during her residence



Fig. 1.—M. H. Seven weeks before death. Characteristic contractures, hyperextension of head, dropped jaw; position indicates condition of spasticity.

owing to her marked psychomotor unrest. From April 26, 1914, to January 14, 1915, the patient showed a gradual decline, physically. She ran a constant temperature of 103 degrees, cried a great deal, had little to say and at times she laughed without apparent cause. By this time there had been distinct changes in her physical condition. Her limbs had become contracted. A general body and limb tremor manifested itself markedly. It was necessary to tube-feed her to maintain nourishment. She continued in this condition and was seen by one of us (F) on February 2, 1915.

Neurological Examination at that time presented the following: The patient was very thin and showed strikingly, at first glance, a dropped jaw with a masked expression of the face. She was easily aroused to emotion. She did not respond to simple or complex commands. Contractures and fixation of all the joints, small and large were manifest (see Fig. 1). She was running a constant temperature of 103 degrees.

Contractures.—Evidently all her joints were fixed and rigid. There was some pain upon any attempt to straighten them out. Her head and neck were fixed and hyperextended. Her jaw was dropped and she could not hold it up, she could not show her teeth by gritting. The fingers were flexed, thumbs turned into the palms of the hands, wrists flexed and averted so that the palms looked outward (see Fig. 1). The arms were abducted, the forearms abducted, due to flexing and fixation at the elbow. The legs and thighs were flexed and fixed. The legs were also drawn up on the abdomen. The feet were turned in and a dorsal extension of both toes was present. Her entire musculature was firm and hypertonic.

Tremors.—There was a general body tremor involving also the tongue and jaw. This tremor was increased by excitement and also when she tried to hold herself up in the bed, under effort. It was quite noticeable when the photograph was taken. It was regular in constancy, but varied in tone according to emotion. When she made an effort there appeared to be a spasm of all her flexor muscles. There were no fibrillary twitchings and no typical athetoid or choreiform movements.

She could not eat due to the spasm of the muscles of mastication (dysphagia). She could not talk due to spasms of the muscles of articulation (anarthria), although at times she could pour forth a most profuse vulgarity (profanity can hardly be called a genuine form of speech, there is no specific appropriateness; they are little more than animal outcries; as such they might survive the destruction of that duty of producing thought with selected words).

There were no cranial nerves involved as far as cooperation would allow. The pupils were equal and regular in outline. They reacted well to light and to distance. The muscular movements of the eyes showed no disorder. The optic discs were negative. There was no nystagmus and no evidence of labyrinthine or cerebellar disease.

She had never vomited nor shown any evidence of heart involvement or autonomic nervous phenomena. At no time had there ever been noted any evidence of liver disorder and physical examination showed no enlargement or atrophy by percussion and palpation. She was unable to protrude her tongue which might have been due to spasticity or to paralysis.

The superficial reflexes of the upper extremities were diminished. The abdominal and epigastric reflexes were absent. The knee-jerks were diminished probably due to the flexion and fixation of the abductor muscles. The right great toe suggested dorsal flexion. There was also an atypical Oppenheim and Gordon great toe response. The left great toe presented a plantar flexion. She could not walk, sit up or coordinate to any degree for the tests for gait. Her attitude and posture were quite characteristic (see Fig. 1).

There was no evidence of sensory disturbances.

Physical examination of the viscera evinced tuberculous processes in both lungs. No tubercle bacilli found in sputum. The von Pirquet test was not made.

The spinal fluid analysis evinced two cells per cubic mm. The Wassermann reaction on both the blood and spinal fluid was negative. Lange's colloidal gold reaction was negative. The globulin reactions by the technic of Nonne, Noguchi, Ross-Jones and the combined method gave negative findings.

At this time the diagnosis had resolved itself into either juvenile paralysis agitans or Wilson's progressive lenticular degeneration. The presence of pyramidal tract symptoms was rather in favor of a central disorder with infringement upon the internal capsule so that at that time a tentative diagnosis of progressive lenticular degeneration was made.

During the next month she was seen several times and further observation led us to conclude the case to be one of actual brain disease with the lesion in the region of the lenticular nucleus. She died March 4, 1915, and the necropsy verified the diagnosis by the finding of a typical cirrhotic liver and a degenerated left lenticular nucleus.

To Recapitulate the Clinical Findings.—A young woman of nineteen years whose physical development was normal and whose mental development was probably one of inferiority (constitutional inferiority) becomes "nervous" at the age of 15 years and has what is called a "convulsion" following which there is noted a tremulousness of the extremities and a later definite involvement of the central nervous system associated with changes in motion and affectivity. Dysarthria and dysphagia developed and a spastic emotional state soon became prominent. Along with all these phenomena of muscular hypertonicity there was, from the beginning of the disease, a coarse rhythmical tremor of the entire body. The neurological examination did not reveal any disturbance of the cranial nerves, there was no evidence of sensory disorder and with the exception of a dorsal flexion of the right great toe there were no signs of a disturbance of the pyramidal tract system, yet, the masked expression, dysarthria, dysphagia and increased motor tonicity were present. Although, the face was spastic and the mouth wide open and to all appearances in a severe grade of mental deterioration, when attempts were made to move her, she watched every movement and assumed more markedly the painful emotional expressions as described by Wilson and Mills. There was no evidence of liver disorder and the temperature could be accounted for by the tubercular processes in both lungs.

AUTOPSY FINDINGS.—The autopsy was performed four hours after death. A general observation of the body showed the prominence of contractures of the extremities. She was greatly emaciated with trophic skin lesions and secondary infection.

Without going into detail regarding the body viscera* suffice it to be stated that areas of tubercular lesions, healed and active, were found in both lungs. The heart was normal. The spleen showed passive congestion and enlargement. There was no affection, grossly, of the kidneys or adrenals noteworthy. There was an infantile uterus and atrophic ovaries. The large intestines, especially the descending colon, showed many confluent tubercular ulcers, none having perforated, however. The thyroid was atrophic, no preservation of the thymus gland. The liver showed a typical nodular cirrhosis with a glossy capsule and which, upon cut section, present a multilobular cirrhosis.

HISTOPATHOLOGICAL SYMPTOM-COMPLEX.—*Liver.*—The appearance of the liver was exceedingly striking and suggested immediately Laennec's cirrhosis. It was somewhat smaller than normal, yet in no way misshapen and weighed

*We wish to express our thanks to Dr. H. S. Bernstein for his report upon the abdominal and thoracic viscera.

1,030 grams. Its consistency was firm and cut on section with marked resistance. The external surface was smooth but gave one the feeling of slight elevations and shallow depressions as the fingers were passed over the surface. Upon cut section there were presented multiple lobulations, varying in size from that of a pin head to that of the little finger nail, varying in shade of color but in no way yellowish to indicate jaundice or green to indicate extravasation of bile. These lobulations were somewhat oval in shape and were separated from each other by strands of connective tissue varying in amount. The gall bladder showed no signs of disorder and there were no stones in the ducts. (See Fig. 2.)

The sections for microscopical examination were stained with hematin and eosin and toluidin blue. The most striking feature was the preservation of liver cells in certain areas, with the sudden appearance of degenerated areas, large in area compared to the normal liver cell areas, and in which fatty degeneration was prominent. Then, again, the appearance around these areas of connective

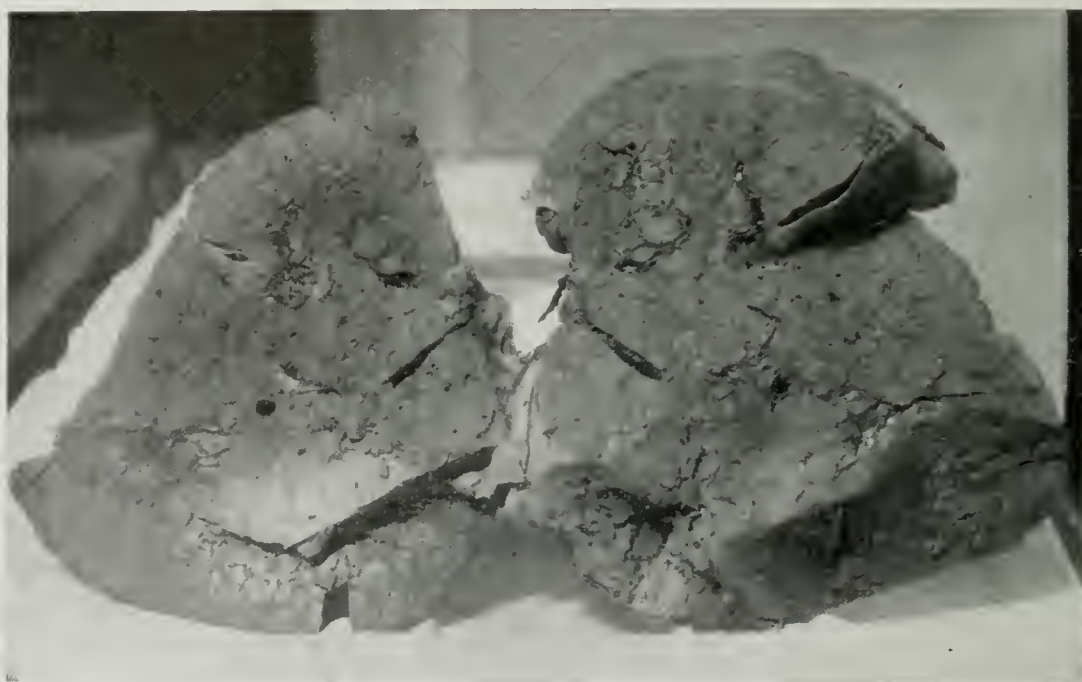


Fig. 2.—M. H. Cut section, showing the whole liver to be cirrhotic. The cirrhosis is multilobular, without being bile-stained and divided by connective-tissue hyperplasia.

tissue hyperplasia, varying in breadth and degree of fibroblastic activity with a moderate infiltration of round cells. Some of the sections indicated by the scarcity of round cells and the relatively few elongated nuclei of the fibroblasts undoubtedly were older processes. In these connective strands were occasionally seen groups of nuclei, arranged in somewhat parallel rows which might indicate new bile capillaries or hypertrophic bile ducts. In some places the strands of connective tissue appeared to invade the lobule and separate the liver cells into columns. There appeared also in certain portions, to be a connection between these so-called new bile capillaries and a column of liver cells. It has been suggested that they might be liver cells reverting to their primitive state rather than new capillaries. In the more degenerated areas necrosis to the extent of fatty infiltration and degeneration was very prominent (see Fig. 3) and the normal liver structure completely losing its cellular character. Throughout many of

the sections liver cells in the process of regeneration could be noted by the changes in the nuclei, double nuclei and a tendency to mitotic formation.

EXAMINATION OF THE BRAIN AND SPINAL CORD.—After the removal of the brain, a general inspection indicated that the left hemisphere was somewhat smaller than the right especially in the frontal region. There was no disturbance in the patterning of the cerebral convolutions. There were no palpable areas of softening or hardening indicating neoplastic growths. The brain was placed in hardening fluid (formalin) and cut when hardened.

Cutting each hemisphere as near as possible through the area suggested by Marie resulted in the following observations (see Fig. 4) :

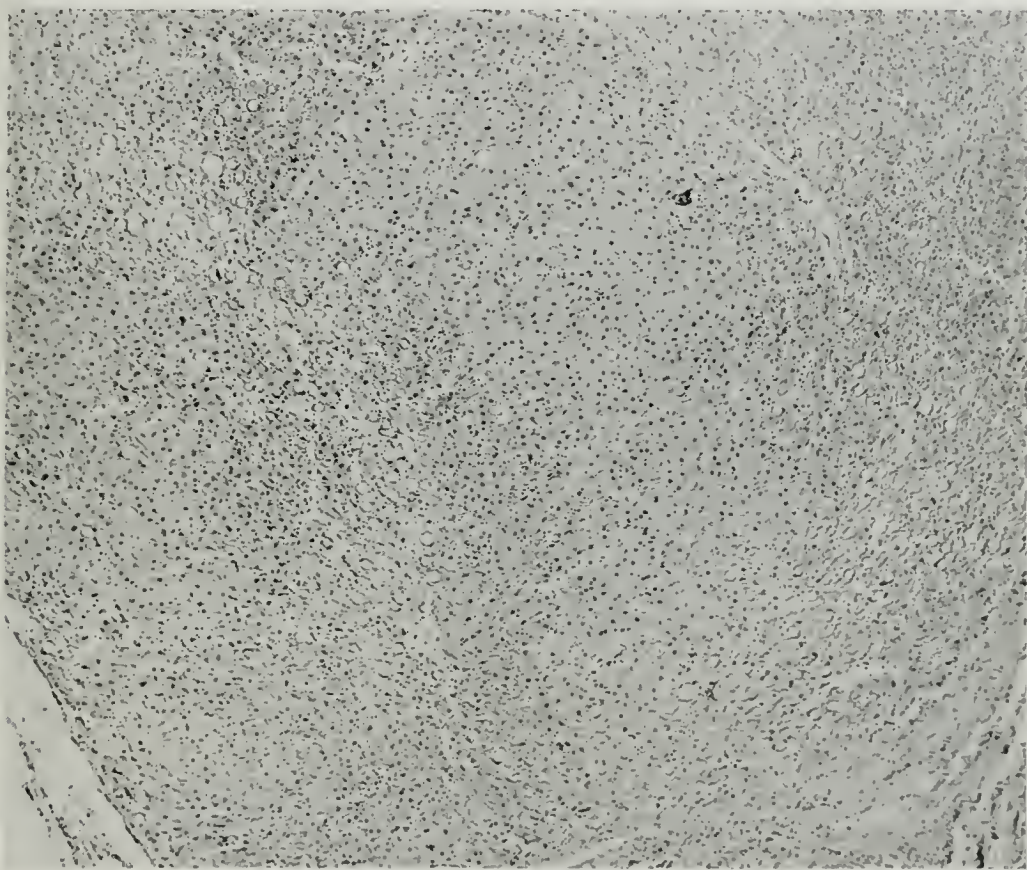


Fig. 3.—Low power of section of liver showing degenerated areas, connective-tissue hyperplasia, preservation of normal liver cells, and capillary formation.

It was first noticed that the markings characteristic of the corpus striatum were lacking on the left side and somewhat less marked on the right side. The left caudate nucleus appeared to be somewhat more shrunken than the right and there was a decided dilatation of the anterior horn of the left ventricle. The optic thalami showed no abnormality. The internal capsule on the left side appeared to be confluent with the tissue that evidently made up the area where the lenticular nucleus was primarily situated. There was no putamen or globus pallidus on the left side, as such and yet there were several small areas which suggested areas of necrosis. On the left side by palpation one noted that there was less resistance than on the right side and that to the finger touch it was soft, as if degenerated. There were no decided cavity formations. The only indication of an outer margin of the original lenticular nucleus was a small uneven broken strip less than a millimeter in width, separating the external

capsule from the degenerated lenticular nucleus. This small broken strip was very small in thickness as well as in breadth and about 1 cm. in length. The caudate nucleus was very much shrunken but still compared with the region of the lenticular nucleus it was well preserved and its fibres could be easily distinguished. A transverse section in the region of the corpus striatum of both hemispheres of that part of the brain removed in the initial cutting along the Marie line of selection, showed very plainly the shrunken caudate nucleus and the dilatation of the anterior horn of the left ventricle.

On the right side the markings were still somewhat distinct and the lenticular nucleus showed a preservation of both the putamen and the globus pallidus. The external capsule was present without disintegration and in the internal capsule, both in its anterior and posterior limbs, appeared to be normal.

The cortex and white matter of the brain in general showed no abnormality. The convolutions in the region of the Island of Reil showed no



Fig. 4.—M. II. Brain. Horizontal section showing almost complete destruction of the left lenticular nucleus and a partial destruction of the right lenticular nucleus; the caudate nucleus being much smaller on the left side with a dilatation of left ventricle.

obvious change or disorder. There was no evidence of internal hydrocephalus, no inflammation of the ependyma and there were no granulations in the fourth ventricle.

The spinal cord showed no abnormality in its macroscopical appearance. Sections of the cord from the cervical, dorsal and lumbar regions showed no disturbance in the markings as far as could be observed. No disturbance was noted in the meninges.

MICROSCOPICAL EXAMINATION.—*Spinal Cord.*—Histopathologically an examination of the various regions of the spinal cord showed no disorder in sections stained with hematoxylin and eosin and toluidin blue. It is unfortunate that in the primary fixing sections were not taken to examine by the Weigart and Marchi methods. With toluidin blue the sections showed

a fairly large number of cells and they seemed to have preserved their normal outline and appearance. The grouping appeared to be also normal. In the cervical region they stained in normal shape and yet a few were seen to be stained much more deeply but without any marked disturbance in the nuclei. Occasionally one would come across a cell which suggested the

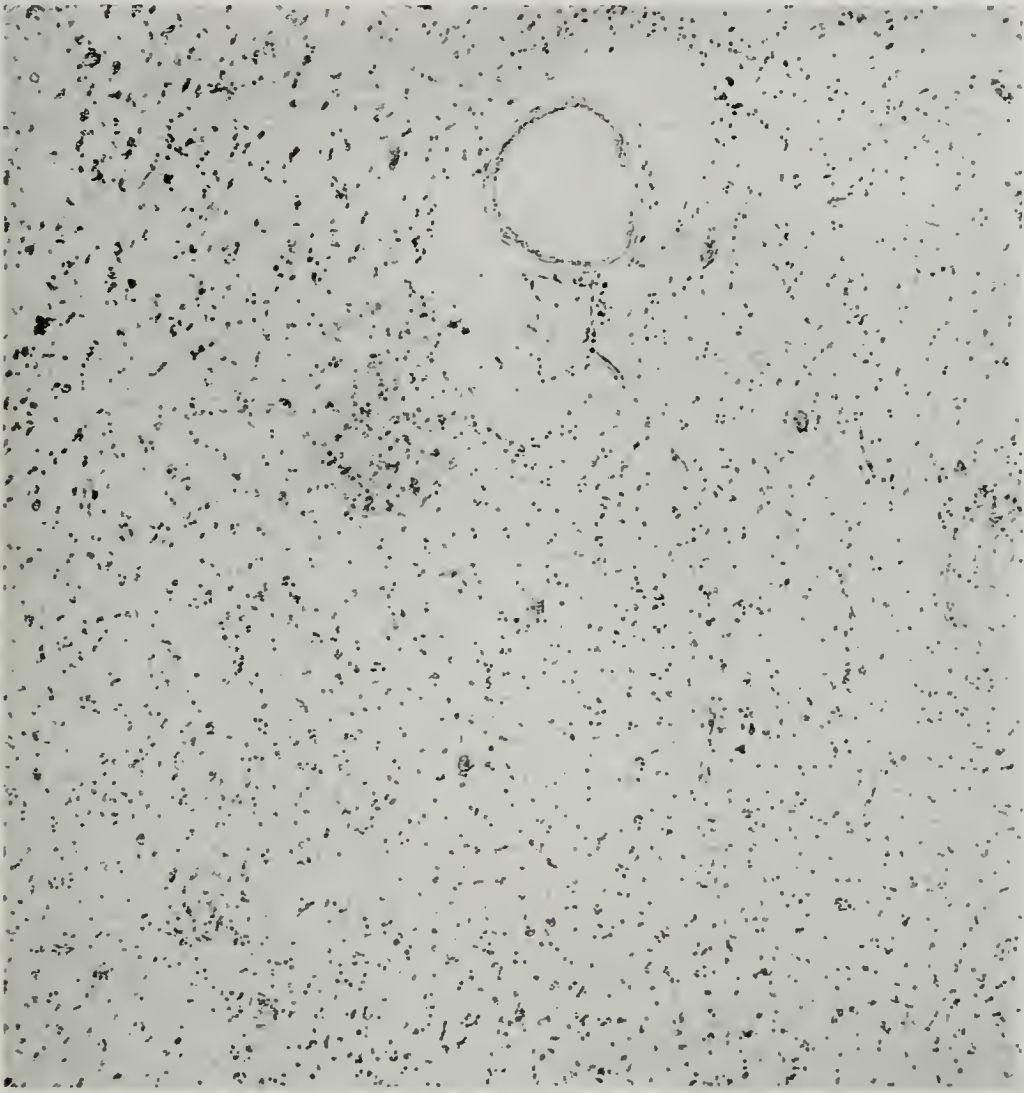


Fig. 5.—Low power of left lenticular nucleus, showing neuroglial increase, scarcity of nerve cells, no sclerosis of vessels but an enlargement or dilatation of Virchow-Robin space.

pyramidal cell in outline and yet was not unlike the pyramidal cells seen in terminal exhaustive infections.

Meninges.—Examination of the coverings of the brain and cord showed in no way abnormality. There was no thickening, no injection of the vessels, no lymphocytic or plasma cell infiltration.

Motor Cortex.—A serial section taken from the right motor cortex showed no abnormality in the arrangement of the cells in their various layers. There was no perivascular lymphocytic infiltration; there was no invasion of the cortex by plasma or round cells and there did not appear to be any inflammatory reaction as indicated by the increase in vascularity, new blood vessel formation or new lymphatic channels.

Sections taken from both the right and left lenticular nucleus evinced the following: *Left.*—The tissue seemed to be composed of a comparatively

thick network neuroglial increase. In various fields it was noted that there were probably reactions such as is seen in satellitosis. The nerve fibers were few with only an occasional nerve cell, which cell was usually misshapen and the nucleus eccentrically situated, the cytoplasm deeply stained but not showing to any marked degree the granulation. The vessels were not scler-

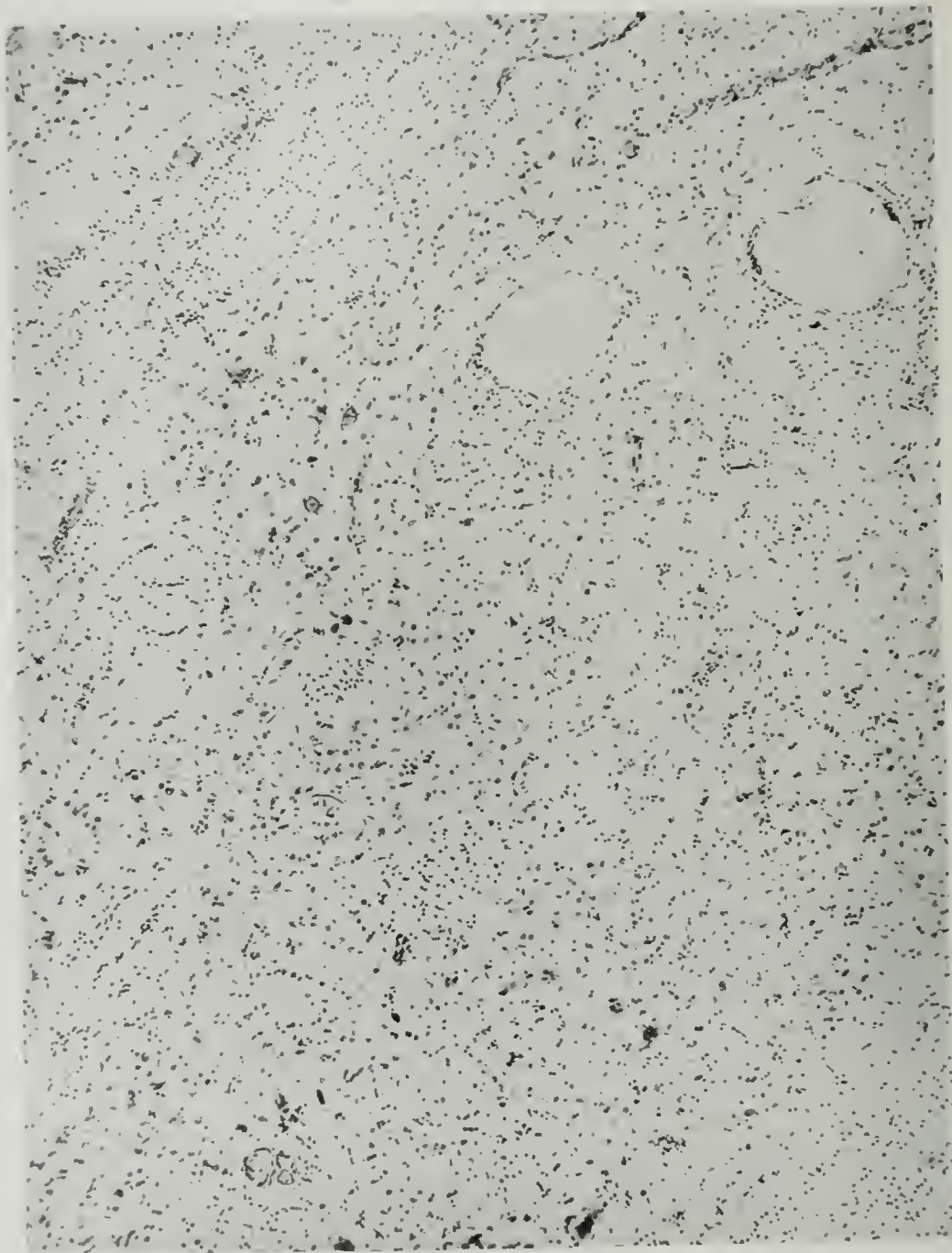


Fig. 6.—Section from left lenticular nucleus showing marked neuroglial increase, degenerated nerve cells in several stages, tendency towards the formation of satellitosis.

osed or occluded. It was not an uncommon observation to see a blood vessel with its thin coats lying in an open space, evidently an enlargement or dilatation of the Virchow-Robin space.

The most marked feature about the section from the left lenticular region was the few nerve cells not in the putamen and the globus pallidus. The caudate nucleus also showed some changes as the glial reaction found in the putamen. The internal capsule stained normally but did appear to

have a connective tissue reaction in the shape of an infiltration of round cells.

Right.—On the right side the reaction did not appear to have reached the stage as seen on the left. Still, there was a decided evidence of sclerotic changes as indicated by the neuroglial increase and the tendency to satellitosis. Under the high power the area seemed to be a mass of neuroglial overgrowth with new vessels and a scarcity of nerve cells, although more were found on this side than upon the left. The neuroglial tissue about the cells appeared

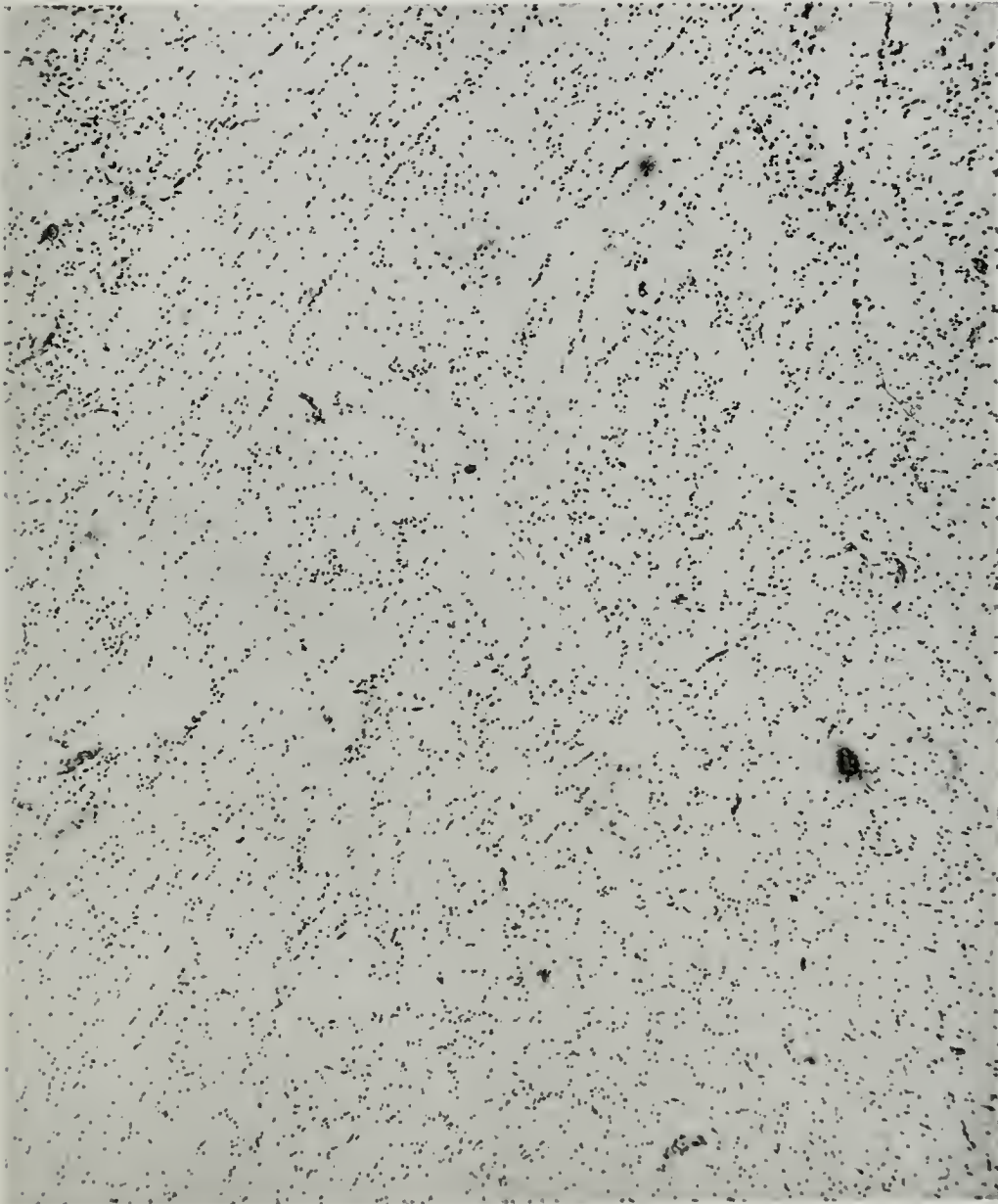


Fig. 7.—Sclerotic changes in the neuroglia are evident and in the upper left-hand corner is an indication of new vessel formation.

to be an active growth. The nerve cells in the region of the globus pallidus were shrunken or swollen and stained very deeply. In some fields the large pyramidal cells appeared to have taken on a coarsely granular appearance in their cytoplasm and had a deeply stained nucleus. About these cells there were frequently seen small elongated cells, probably the inflammatory neuroglial reaction. (Figs. 5, 6 and 7.)

To Recapitulate the Pathological Findings.—It is evident that the upper

motor neuron is in its entirety in no way involved in this disease except secondarily due to the infringement of the neorogial overgrowth upon the internal capsule on the left side. There is no disturbance in the motor cortex and no disturbance in the cells of the anterior horns. The microscopical changes in the lenticular region appear to consist in a glial overgrowth which in some places has disintegration, in some places in the dense glial overgrowth an occasional pyramidal cell will make its appearance. There is occasionally seen a cellular infiltration suggesting a satellitosis. The vessels are not sclerosed or occluded but it is frequently noted that the Virchow-Robin space between the vessel and the surrounding tissue is dilated to a considerable degree. The pyramidal cells are scarce in number on both sides, more marked loss on the left side and those which are present show either a partial or complete cellular disintegration as indicated by the cytoplasm and the nucleus. The entire reaction appears to be that of a replacement gliosis that has not gone on to complete disintegration with the formation of cavities although there are sections which indicate an advanced stage of necrosis.

Diagnosis.—Hughlings Jackson once said “positive signs could not be caused by negative lesions.” The clinical symptoms, tremor, spasticity and emotional instability are essentially positive symptoms and one must therefore conclude that they are caused by a destructive lesion. In this case the destructive lesion appears to confine itself entirely to the lenticular nucleus and the caudate nucleus upon the left side, as far as the pathological examination has been able to point out. It is Wilson’s view “that destructive lesions of the lenticulo-rubrospinal tract remove the normal inhibition or steadying influence which the corpus striatum is supposed to exercise upon the anterior horn cells.” As a result of this removal the steady innervation of the anterior horn cells is impaired and the more the pyramidal tract is innervated the more obvious does the tremor become. This same explanation may be applied to the influence exerted by the cortical cells upon the pyramidal tract system and cause the development of the spasticity and hypertonicity.

The lenticular syndrome as described by Wilson is similar in many respects to that described in this case, yet, there is in addition an involvement of the right pyramidal tract system which might be interpreted as an extension of the gliotic process. However, it seems best to present the case clinically and pathologically, as one of progressive lenticular degeneration of Wilson’s type.

We wish to express our indebtedness to Mr. William S. Dunn, of Loomis Laboratory, Cornell Medical University, for his work upon the photomicrographs.

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SPECIFIC PARENTERAL DIGESTION AND ITS RELATION TO THE PHENOMENA OF IMMUNITY AND ANAPHYLAXIS*

BY J. BRONFENBRENNER, PH.D., PITTSBURGH, PA.

WHILE it is generally observed that repeated injections of toxins or toxic bacterial proteins render the organism resistant against even a multiple lethal dose of those toxic substances, experiments have shown that under certain conditions the reinjection of even absolutely inoffensive substances may produce very severe phenomena of intoxication and even death of experimental animals.

It is this exaggeration of toxicity which is the most striking phenomenon in anaphylaxis.

"Anaphylaxis is a reverse of vaccination; anaphylactic animals react to the second injection much more strongly than to the first, which is the more surprising because, in the majority of cases, the substances used are not toxic even in very large doses," wrote Besredka in 1908.

Where does this increased toxicity come from?

Since the substances injected may by themselves be inoffensive to normal animals, even in very large doses, it is evident that conditions in the body of anaphylactic animals must be responsible for the change in tolerance to protein.

Why is it then, that in the one case, the preliminary injection of protein raises the resistance of the animal to the subsequent introduction of the same protein, whereas in the other case it destroys even the natural tolerance of the animal, making it incomparably more vulnerable than before?

It is this *loss by the animal of its normal degree of immunity*, or resistance to the parenteral introduction of foreign proteins, which suggested to Richet the name of "*Anaphylactic*" for this state of hypersensitiveness of the experimental animal, following a preliminary inoculation with the foreign substance. The very name given to this phenomenon by Richet, who was one of the first investigators in this field, shows that the nature of the anaphylactic state was assumed to be quite the opposite of that of immunity, or heightened resistance, in spite of the fact that both conditions seem to be brought about in the experimental animal by a very similar procedure.

It is because the parenteral introduction of foreign protein may lead in one case to immunity, and in the other to hypersensitiveness, that some authors suggested as a working hypothesis, that the introduction of foreign protein (antigen) may lead to the formation of two independent sets of antibodies, one responsible for the heightened resistance, and the other for the heightened vulnerability.

These earlier theories, however, did not find confirmation in subsequent investigations.

*From The Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.

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At present the consensus of opinion among workers in this field of scientific endeavor, seems to point to the assumption that, in the measure in which the development of new properties in the blood of experimental animals is responsible for the establishment of either heightened or diminished specific resistance, the same set of substances is supposed to confer these outwardly contradictory changes in tolerance of animals to reinjection of foreign proteins.*

There remains, however, an open question as to the nature of these new properties of the blood serum, as well as to the mode of their action. Still less definite is the information as to the reason why these changes, brought about in experimental animals by the parenteral introduction of foreign protein, should produce a seemingly opposite effect on the natural tolerance of animals to these substances.

The changes in tolerance to a given protein, following a single or a multiple systematic introduction of the same protein into experimental animals, have been studied and variously interpreted by different investigators.

But for very slight minor differences, all the theories suggested can be brought back to two fundamental theoretical conceptions.

One, assuming after Ehrlich, that the foreign protein, introduced parenterally into the experimental animal, is anchored by such cells of the body, as happen to possess chemically active radicals (receptors), with specific chemical affinity for corresponding chemically active radicals of the protein introduced (antigen). The union of the respective radicals in virtue of their specific chemical affinity, is followed by the neutralization of the chemical avidity of the cell concerned, and by the subsequent restoration and overproduction by the cell, of the radicals thus saturated by antigen. This phenomenon (studied and described by Weigert), leads finally to the condition in which the cell finds itself overburdened with the radicals in question and at a certain time during the process, such a cell casts off into the circulation the excess of these chemically active radicals. Such substances, appearing in the circulation as a response of the cells to the introduction of a foreign body, are known under the name of antibody. The antibodies, according to this view retain, while free in the circulation, their power to combine with, and anchor the homologous protein, when such is reintroduced later.

Whereas the anchoring of antigen by the specific receptors of the cell is followed by incorporation of such antigen into the body of the cell, by virtue of the general digestive mechanisms of the cell, the detached receptors, circulating in the blood stream, combine with such specific antigen without digesting it, but merely changing such antigen in a manner in which it becomes more easily attackable by leucocytes, as well as by the complement, which is supposed to be the active principle directly responsible for "cytolytic" as well as "albuminolytic" properties of the blood serum.

According to the other fundamental theory, represented best by the work of Vaughan and his collaborators, the parenteral introduction of foreign protein stimulates the production of specific ferments within the body of experi-

*These views of course do not exclude the role body cells may have in the mechanism of specific protection.

mental animals. Such ferments, circulating in the blood stream, attack and digest directly the homologous protein when it is reintroduced later.

Although the above conceptions differ very substantially from each other on the question of the intimate nature of mechanisms governing the physiological processes, following the introduction of foreign protein into the animal body, both theories, in fact, indicate that such foreign protein undergoes parenteral assimilation, or digestion in the broader sense of the term, and thus eventually is eliminated from the circulation.

It is this element of ridding the body of the foreign material which is responsible for the view of the whole process as a protective mechanism, especially, because practically all the protein substances, when introduced parenterally, are apt to exhibit different degrees of toxicity, if the amount injected is sufficiently great.

Whichever of the two views upon the mechanisms of parenteral digestion is correct, the careful observers beginning with Jenner in 1798 and Magendi in 1839, have noticed repeatedly, that parallel with the apparent development of specific protective mechanisms, the parenteral introduction of protein foreign to the body, may give rise also to other mechanisms, detrimental to the well-being of the animal.

According to the views of the school following in the footsteps of Ehrlich, the reason for such paradoxical action of parenteral introduction of protein, is the fact that, during the extracellular specific lysis of formed, as well as unformed protein antigen in reinjected animals, this antigen is broken down, through the action of complement, and the poisons preexisting in the unaltered antigen are thus liberated.

Such a view has been especially plausible since Vaughan has shown that any protein can be broken up by chemical means, so as to yield a very powerful poison.

Friedberger, who deserves especial credit for most of the work in this direction, has shown that such poisons could be produced in the test tube by imitating the specific lysis, supposedly taking place in the body. In his earlier experiments Friedberger obtained from normal guinea-pig serum, which had been allowed to stand for some time with the washed specific precipitate (formed by rabbit serum immunized against sheep serum with the serum of the latter), very strong poisons which killed the guinea-pigs instantly with symptoms of acute shock. He named the poisonous substance anaphylatoxin, and assumed that it arose from the digestion of the specific precipitate, by the ferments of normal guinea-pig serum (complement).

According to the views of Vaughan and his followers, toxic phenomena in anaphylaxis are due to the split products of direct digestion of antigen, by the specific ferments present in the blood and tissues of sensitized animals.

More recently there has appeared a great number of publications concerning the question of the mechanism of the specific parenteral digestion. Abderhalden and his collaborators who are primarily responsible for this work, have taken up the theory of specific ferments and, by using specially devised methods, have seemingly demonstrated *in vitro* the presence of such ferments in the blood of prepared animals.

These findings, if correct, seem to offer a valuable link in the chain of reasoning of the earlier supporters of the theory of specific ferments.

Approaching the question of the mechanism of anaphylaxis at this stage of its development, the investigator is confronted with a fundamental question: Assuming that the anaphylactic shock is due to the liberation of poison in the body of sensitized animals, through the specific cleavage following the reinjection of antigen, what is the mechanism of this cleavage?

In order to answer this question, one must first determine the nature of changes taking place in the normal physiological processes of the animal, following the first experimental parenteral introduction of the foreign protein.

On the one hand, the hypothesis of Ehrlich suggests that such parenteral introduction of foreign protein is followed by the production of *specific antibodies*. On the other hand, Vaughan and his followers, without definitely denying the production of antibodies, assume that parenteral assimilation of foreign protein is followed by the output of specific ferments. In fact Abderhalden states definitely, that the parenteral introduction of foreign protein is followed by the production of specific ferments *parallel* with the production of specific antibodies, but independent of the latter.

If the interpretation of the phenomena observed by Abderhalden is correct, the production of specific ferments seems to be an even more general mechanism than that of production of antibodies. For antibodies have been thus far demonstrated only in cases of parenteral introduction of substances of animal or plant protein origin, provided that this protein is foreign to the species. Specific ferments, however, are claimed to have been demonstrated not only upon the parenteral introduction of such substances, but also upon that of proteins of homologous, and even autogenous nature, provided these substances are foreign to the blood ("blutfremd"). Moreover, the parenteral introduction of substances like gelatin, pepton, cane sugar, or casein, is claimed by the Abderhalden school to produce specific ferments, capable of attacking said substances, both *in vitro* and *in vivo*. Thus the group of substances which can play the part of antigen in the production of antibody, is included in that of the substances capable of causing the production of specific ferments, but is only part of it.

Such a conception of parenteral digestion, on first examination, seems very useful as a working hypothesis, for, if it is possible to prove the presence of such specific ferments, all findings from the realm of immunity can be brought into the sphere of biochemistry. The just criticism which the chemists had for a long time against the conception of immunologists, who dealt with "antibodies" and "alexins"—terms which seem to name, but not define, the still unknown substances,—would be immediately answered.

It is thus, that a number of men, familiar with phenomena of immunity, and anxious to find the general physiological principles underlying this very important mechanism, have turned their attention to the possibilities offered by the methods of investigation enunciated by Abderhalden.

In repeating the work of Abderhalden and his collaborators in this laboratory, however, we came to doubt their conclusions. Our experiments have shown that, although under certain conditions of experiment, the antigen may

be actually digested by the ferments of the blood of prepared animals, the ferments responsible for such digestion are not specific. In fact, as others have shown before us, the digestive ferments are present in every fresh serum, and if set free, can digest any suitably prepared substratum *in vitro*, without any specific predilection.

According to the findings of a number of investigators, the ferments normally present in the serum are usually inhibited by a simultaneous presence in the serum of antiferments. The removal of the latter liberates the active ferments and allows digestion to take place. Indeed Bordet, Nathan, Plaut, Flatow and others have demonstrated quite definitely, that mere mechanical adsorption by any of the substances like agar, kaolin, starch, and the like, may free serum of its antiferments and thus liberate the normal ferments.

On the other hand, as Schwartz, and more recently, Jobling and Peterson have shown, serum lipins may also inhibit the activity of normal ferments of the serum and their removal or (as we found it) even change in their solubility, may thus result in activation of the normal nonspecific ferments of the serum.

It is thus that a number of authors tried to explain the digestion, accredited by Abderhalden to the activity of newly produced specific ferments, by assuming that it was due to the mechanical adsorption of antiferments by the substratum and subsequent liberation of normal, nonspecific ferments.

Our own experiments, however, convinced us that such is not the case. We found that although the ferments concerned in the digestion are not specific (inasmuch as they can be made to digest any substratum), the phenomenon, as a whole, is not devoid of a certain degree of specificity and therefore could not be reduced to mechanical adsorption of antiferments.

Our systematic study of this question brought us to the conclusion that the element of specificity lies, not in the ferment itself, *but in the mechanism of its activation*. Namely, we found that the apparent specificity of ferment action *in vivo* assumed by Vaughan, and demonstrated *in vitro* by the methods of Abderhalden, is due to the fact that the combination of specific serum with its corresponding antigen, *in vivo* as well as *in vitro*, is followed by a radical change in the degree of dispersion of serum colloids. This physico-chemical change in turn is followed by the activation of a normal, nonspecific ferment of the serum.

If such a conception is correct, then the phenomenon of specific parenteral digestion may be explained on a basis very similar to that offered by the chemical theory of Vaughan, but instead of specific ferments, the digestion will be ascribed to normal nonspecific ferments, present in any fresh serum (complement?) and set free by a specific mechanism of combination between the antigen and antibody, very similar to that recorded by the meiotagmin reaction of Ascoli.

As was mentioned above, the toxic phenomena in anaphylaxis are ascribed by the chemical theory to the action of the split products of antigen, attacked by the active principle of the serum of the sensitized animal.

This hypothesis is based on observations, that cleavage of the antigen *in vitro* by chemical means yields poisonous split products; that combination of specific serum with its corresponding antigen yields toxic substances, identical

in their physiological action with those produced from the antigen chemically; and lastly the actual cleavage of antigen by the ferments of specific serum, can be demonstrated *in vitro*.

There exists, however, another view of the origin of so-called anaphylatoxin. This view represented by the work of Sachs, Nolf, Doerr, Ritz, Bordet, and others is known as "physical theory." In the main this theory suggests that the source of anaphylatoxin is not the antigen, but the protein of the serum itself.

As mentioned above, in his original experiment, Friedberger has succeeded in producing the toxic substances *in vitro* by a process similar to that which was supposed to be responsible for the anaphylactic shock *in vivo*. Namely, he allowed antigen to combine with its specific antibody and added the complement to this mixture. The poisons obtained in this manner *in vitro*, when injected into normal animals, were able to produce typical anaphylactic symptoms.

In his later experiments, however, Friedberger succeeded in obtaining similar poisons from bacteria and other proteins by their incubation with normal guinea-pig complement without the concurrence of specific antibody. He still believed, however, that in these experiments also the poison was derived from the protein of the substratum. Even when Bordet, Nathan, Mutermilch and others have been able to obtain similar poisons by the incubation of normal guinea-pig serum with agar, starch, or kaolin, and have decided therefore, that the poison must originate from the serum, Friedberger still objected to these conclusions on the basis that these substances may contain a small amount of protein impurities, which really furnish the substratum for the formation of anaphylatoxin.

In studying this controversy one finds two really independent questions involved in it. The first question seems to be: "What are we to take to be the anaphylatoxin?" It is only after agreeing as to the exact meaning of this term, that one can attack the main question: "Which is the source of poisonous substances in the anaphylatoxin formation—is it antigen or the serum?"

Originally, the term "Anaphylatoxin" was applied by Friedberger to designate the poison which was supposed to be identical with the one produced *in vivo* during the anaphylactic shock. This poison had two essential characteristics, making it possible to assume its identity with the substance causing anaphylaxis. First, the method of its production from combination of antigen with antibody and complement, and second, its physiological action upon normal animals, which is identical with anaphylactic shock.

In discussing the question of the origin of anaphylatoxin, different authors apparently used as criterion in their terminology only the second characteristic of the original anaphylatoxin—namely, its physiological effect upon normal animals. Thus, many authors have called anaphylatoxin the chemical poisons obtained by Vaughan from bacteria; Nathan, Bordet and Mutermilch called anaphylatoxins substances obtained by them from the serum by adsorption with inert substances, etc. Attempts of these authors to explain the nature of anaphylatoxin seem to be generally inadequate, because, even though, as my experiments have also confirmed, by digestion of serum with kaolin, for instance,

one can produce from serum a substance which is similar in its physiological action, on the one hand, to the original anaphylatoxin of Friedberger, and, on the other hand, to the chemical poison of Vaughan, its identity with one or the other is not proved by this similarity alone. The identity between all these substances has to be proven before the experiments above could be taken into consideration in discussing the probable nature of anaphylatoxin.

This we attempted to do in a series of experiments in which we studied the actual mechanism of the production of poison in each instance. These experiments have shown that in the poisons arising during the incubation of the serum with kaolin or starch, as well as those arising when specific serum is digested with its antigen, the serum is the source of poisonous products. Moreover, it was found that the products of such autodigestion of serum are toxic only to homologous animals. This would indicate that during the anaphylaxis *in vivo* the toxic split products originate only from the digestion of serum or tissues of the animal and not from heterologous protein of the antigen.

Since it is established that in the experiments of Bordet, as well as in those of Friedberger, the formation of toxic split products is identical, namely, that it is due to the digestion by the normal serum ferments of autogenous protein, the similarity of the biologic properties of the respective end-products of such digestion may speak for their identity. On the other hand, the fact that in Vaughan's experiments the poison arises from heterologous protein shows that this poison is not identical with anaphylatoxin, in spite of the similarity of its biologic action upon animals.

These findings, taken in connection with the results obtained by us in the study of the mechanism of specific parenteral digestion, referred to above, suggest that the nature of anaphylatoxin is as follows: Fresh serum contains normal proteolytic ferments whose digestive action *in vivo* as well as *in vitro* is inhibited by the simultaneous presence of some antitryptic elements. This anti-trypsin can be removed from the serum *in vitro* by two independent processes: one, nonspecific,—a simple mechanical adsorption by means of excess of some organic as well as some inorganic substances; the other, specific,—an inactivation of the antitryptic properties of the serum, taking place as a result of the physico-chemical changes in the serum, induced by the specific interaction between the antigen and the antibody of the immune serum. The removal of the inhibiting antitryptic action of the serum, by either method, is followed by the restitution of the activity of the normal proteolytic enzyme, which may attack both the protein of the antigen, as well as the protein of the serum itself. At a certain stage of this autodigestion the split products of the serum protein exhibit toxic properties. Biological properties of these toxic substances indicate their similarity to the anaphylatoxin and suggest that the anaphylatoxin of Friedberger, whether occurring *in vivo*, or produced *in vitro*, is a result of the autodigestion of serum, and not of the protein outside of the serum.

Our findings as described above showed that there is no experimental evidence of the existence of specific ferments, and that the phenomena of parenteral digestion, ascribed by Vaughan to the activity of specific ferments, can be plausibly explained and experimentally demonstrated, without assuming the existence in the body of a special specific mechanism outside of antibody formation.

Having thus answered the first question which we set out to study, we will try to answer the second one.

Namely,—assuming the above mechanism of parenteral digestion to be correct—why then in some cases, does the introduction of protein into normal animals result in the establishment of heightened resistance, whereas in other cases it leads to heightened vulnerability?

As we have stated above, the activation of normal ferments present in specific sera, through the changes in colloidal dispersion following the union *in vivo* between the antigen and antibody, may lead to digestion of antigen as well as to that of the serum itself. When the antigen mainly is digested, the phenomenon is interpreted by the observer as that of protection. When the serum or other autogenous elements are digested, the autogenous split products being toxic to the animal, the intoxication occurs and the observer interprets symptoms as a sign of heightened vulnerability.

It is evident from the above, that the actual mechanism of parenteral digestion in the body of the animal previously injected with the homologous protein, and thus possessing the circulating specific antibody, is the same in both cases. Namely,—the antigen upon its reintroduction is anchored by antibody, and this union leads to the liberation of normal proteolytic ferments of the serum. It is only at this point that the difference may come in, depending upon which substratum is mainly attacked by this ferment.

There must be at this point, therefore, the main problem of the situation. What determines the direction of the activity of the ferments? Is this activity selective and directed exclusively toward sensitized antigen in one case, and the serum protein in the other, or is the difference in two cases only quantitative and not qualitative?

Our experiments point to the second, as a correct answer.

It is true, some of our findings seem to show that sensitized antigen is more readily digested by the ferment in "*momentu nascendi*" and thus there might be a degree of selective action on the part of a ferment. But experiments in this direction are very difficult and the amount of work actually done is not yet sufficient for us to definitely claim such to be the case.

On the other hand, and in the majority of experiments, there seems to be no selective action on the part of the ferment. Both the serum itself and the antigen seem to be equally subject to the attack.

What then determines the degree of toxicity developed during this process of parenteral digestion?

First of all, of course, the amount of the autogenous toxic split products liberated during digestion. And this seems to be in direct relation with the amount of antigen introduced.

It is very well established experimentally that, no matter how small it may be in certain cases, the dose of antigen introduced into a sensitized animal must be sufficient to produce the anaphylactic shock. If the amount of antigen is too small, the shock does not take place, although the experimental animal may show other, milder symptoms, due to partial intoxication.

It is thus evident, that the amount of antigen reintroduced into a sensitized animal, is a very important factor in determining the degree of intoxication.

On the other hand experiments have definitely shown, that exposure of sensitized animals to cold or starvation may reduce the toxic effect of the injection of a lethal dose of antigen.

These experiments suggest, that not only the actual amount of antigen introduced, but also the rapidity with which it unites with the antibody, may influence the rapidity of liberation of ferments and resulting intoxication.

This relation may be still better demonstrated by the known fact, that if instead of injecting at once a lethal dose of antigen into a highly sensitized animal, one would inject the same and even a larger amount very slowly, the acute anaphylactic shock might be averted. Thus, the degree of toxicity developed during parenteral digestion in sensitized animals is apparently determined by the amount of digestion taking place in a unit of time and this in turn depends on the amount of antigen injected in a unit of time.

Such a view of the phenomenon would explain, it seems, how it is that parenteral digestion, following reintroduction of antigen, may at times seemingly protect the animal, whereas at other times injures its well-being.

If the amount of antigen reintroduced into a sensitized animal is small or introduced very slowly, the amount of ferment liberated in unit of time, is very slight, and the amount of autogenous split products may be so small, that the animal may show no apparent symptoms of intoxication. If, however, the amount of antigen is sufficient and if it is introduced rapidly, the rate of activation of the ferment is great, and the animal succumbs to intoxication with autogenous split products.

In case of natural reinfection with pathogenic microorganisms, the amount of antigen, which penetrates into the body of sensitized animals, is usually small enough, and even if it multiplies in the body at the beginning, the process is so slow and the extent of autodigestion in a unit of time is so slight, that the reaction occurs without the visible injury to the host, and this is why the observers called the process "*protective*" and the animal "*immune*."

If, however, the same animal be artificially given a large dose of the same antigen, large amount of ferment is liberated rapidly, causing anaphylaxis and we call the same animal "*hypersensitive*."

However, the amount of the antigen and the rate of its introduction into sensitized animals is apparently not the only mechanism controlling the rate of parenteral digestion, as judged by the appearance of toxic split products.

It is observed for instance, that animals, receiving systematic injections of toxic foreign protein *at short intervals*, can develop their resistance to the given toxic substance to such a degree that the amount of protein containing several toxic doses of antigen, may be injected, no matter how rapidly, without producing even the slightest symptoms. It is noticed, however, that a similar animal inoculated with a similar dose of antigen, the only difference being that between the time of the last serial injection and the test injection sufficient time has elapsed—will succumb with anaphylactic shock.

In both cases the amount of antigen and the rapidity of injection being the same, it is evident that the difference in time elapsed between the last serial

injection and the test injection, determines the difference in the effect of the respective test injections.

Just what happens in the sensitized animal during this period of time, called "incubation period," is differently explained by different authors. Our own experiments lead us to believe that the reason for the difference in the response of the sensitized animals to reinjection in the two cases cited above, is as follows:

The union between the antigen and antibody, as we have suggested above, reduces the inhibiting power of the serum-antitrypsin, and thus liberates its normal ferments. Such can be the case only as long as the remaining amount of antiferment is not too great to interfere with the action of the ferment, as it is liberated. If the amount of antiferment is too great, it may delay, or altogether prevent, the digestive action of the ferments.

When antigen is introduced parenterally into a normal animal, once or in serial injections, such antigen is digested. It is known that the products of such digestion are strongly antitryptic. It is thus, that as the digestion progresses, there is going on all the time the formation of new antitryptic split products, which are eventually either assimilated by the cells in the constructive process of the body, or are eliminated. Such removal of excess of antitryptic elements is, however, not rapid, and as long as the antitryptic split products of the antigen from the previous injection remain in circulation, the rapid action of newly liberated ferments is prevented, and anaphylaxis does not occur. If, however, enough time is allowed to pass, usually about twelve to fifteen days, before the test injection is given, the excess of antitryptic products of previous parenteral digestion are removed, and a rapid action of ferments is thus made possible. Such rapid digestion may cause the shock.

That this view is correct also follows from the analysis of the phenomenon of so-called *vaccination* against anaphylaxis.

It was my good fortune to study the phenomenon of anaphylaxis in Besredka's laboratory when he found that, if the treated animal should be given a second injection of antigen before the expiration of full incubation time after the first injection, such an animal does not respond with anaphylactic shock to the test injection at the time expected (thirteen to fifteen days after the first injection), but later. Moreover, the larger the dose of antigen injected, the longer is the state of hypersensitiveness delayed, and the period of resistance prolonged.

This phenomenon suggested to Besredka the possibility of using it, in order to prevent the undesirable reaction in anaphylactic animals.

Namely, he succeeded in preventing anaphylactic shock in animals having passed the incubation period after their first (or after their last serial) injection, by merely injecting a sublethal dose of the antigen some short time previous to the following test injection of several lethal doses of antigen.

This experiment is the basis of the method, which is used every day now for the prevention of serum sickness in children receiving more than one injection of diphtheria antitoxin.

The mechanism of such "*vaccination against anaphylaxis*" was found by us to be that of retardation of ferment action by the circulating split products

remaining from the digestion following the vaccinating injection of antigen, and not to exhaustion of antibody, as assumed by others.

Such a state of seeming resistance to anaphylaxis, or as it is called "*the state of antianaphylaxis*," was produced by many other procedures than vaccinating injection of antigen.

Thus, it was found that administration of anesthetics, sedatives and many other toxic substances may prevent the subsequent development of anaphylaxis. The mechanism of such action of the substances just referred to, was never adequately explained.

Having convinced ourselves that in the case of "*vaccination*" against anaphylaxis the mechanism involved was that of production of antitrypsin, we tried to see if the same could also be true in the other cases. Actual measurements have shown this to be the case. We found that without exception, the administration of substances known to have the effect of preventing the anaphylactic shock, is followed by a more or less marked increase in antitryptic properties of the blood.

Thus we suggest that administration of poisons, causing destructive changes in the cells of the body, in quantities not sufficient to kill the animal outright, is followed by the death of the tissues immediately affected by the poison. With the death of tissues the intracellular ferments are set free. These ferments, possibly with the collaboration of the ferments thrown out from the surrounding fixed cells, as well as from the blood serum and leucocytes, proceed to dispose of the dead material.

Some of the protein split products of such digestion, together with some of the nonprotein constituents of the destroyed cells, may exert antitryptic action. If a sensitized animal is subjected to such treatment, previous to the test injection, and if such test injection is given before the antitryptic split products referred to above are eliminated, they may retard or stop the activity of proteolytic ferments liberated upon the introduction of antigen, and thus prevent the anaphylactic shock.

The view of specific parenteral digestion, as outlined in the earlier part of this paper offers, it would seem, a plausible basis on which the various seemingly contradictory phenomena of immunity can be satisfactorily explained. Such a hypothesis suggests first of all, that the terminology used in connection with the study of the reactions of the living organism, following its invasion by biological poisons, is not adequate.

In considering the question in the light of its historical development, one is impressed by the fact that ever since the first observations on this subject, the apparent increase of tolerance to biological poisons, following their repeated introduction into experimental animals, has been accepted to be the expression of nature's protective force. This *teleological* conception of the reaction on the part of animal organisms upon the parenteral introduction of foreign material, however, could not adequately explain all the phenomena observed. The work of Vaughan especially has suggested that in spite of the difference in the final effect, the reaction of the animal body must be the same in case of heightened as well as diminished resistance to poisons.

Although our experiments fully confirmed this view, the intimate mechanism of this reaction seems to be different from that suggested by Vaughan.

There seems to be no evidence of the existence of specific ferments and the apparent specificity of digestive processes seems to be due to the presence in sensitized animals of specific antibodies in the sense of Ehrlich.

As to the difference in final effect following the specific activation of serum ferments—it is due to the difference in rate of digestion.

Thus phenomena “immunity” or “anaphylaxis” can be nothing but different forms, in which a greater and more general process of specific parenteral digestion expresses itself to the observer.

THE SEROTOXIN OF JOBLING*

BY N. R. SMITH, A.M., ANN ARBOR, MICH.

THE production of a serotoxin by treating homologous and heterologous sera with chloroform and ether as reported by Jobling¹ has found wide acceptance among workers in the field of anaphylatoxins and is incorporated in the literature of the subject alongside the earlier pioneer work of Richet, Bordet, Friedberger, Nathan and others. Jobling maintains that the ferment action of the serum is held in abeyance normally by an unsaturated lipoidal antitryptic substance; the removal of which by lipid solvents permits autolysis of the serum proteins, thereby forming a poison, which is in all probability identical with Vaughan's protein split product.

Some work was under way in this laboratory in which splitting of germ substance by sera was the desired end. In the light of Jobling's work it seemed reasonable to assume that the splitting action of the sera could be greatly increased and accelerated by shaking out the sera with chloroform before incubation with the germ substance. Therefore rabbit serum was shaken with one-tenth its volume of chloroform four minutes, then centrifuged at 8,000 R.P.M. for ten minutes and the supernatant serum carefully pipetted off from a precipitate that, upon centrifugation, was interposed between the chloroform on the bottom and the serum above. More will be said later concerning this precipitate. As a matter of routine, controls were made by testing the action of the serum on guinea-pigs before incubation with the germ substance. As high as 9 c.c. of the normal untreated serum had been injected without effect, but since the M.L.D. of serum and germ substance after incubation was known by trial to be 3 c.c. or less, the control injection of the normal serum was usually limited to 3 c.c. But upon the injection of the chloroformed serum in 3 c.c. quantities as a control; that is, without incubation with germ substance, the pigs died instantly. Reduction of the dose to one cubic centimeter still produced death in most cases, and always a marked prostration. A summary of these observations is given in table I.

*From the Hygienic Laboratory, University of Michigan.

TABLE I.

Rabbit serum shaken at room temperature with one-tenth volume of chloroform, centrifuged ten minutes at 8,000 R.P.M.

FIG.	DOSE.	RESULT.
1	3 c.c.	Instantaneous death.
2	2.5 c.c.	" "
3	2 c.c.	" "
4	1.5 c.c.	Prostration; died in four hours.
5	1 c.c.	Instantaneous death.
6	1 c.c.	Deep prostration; lived.
*7	3 c.c.	No effect.
*8	3 c.c.	No effect.

*Controls; received normal untreated serum.

The character of the deaths, together with the autopsy findings clearly indicated atypical anaphylaxis and pointed strongly to the *residual chloroform* in the serum as the toxic agent. The serum was perfectly clear and free from emulsion by reason of the thorough centrifugation, but had a faint odor of chloroform. To determine roughly the influence of the residual chloroform, a portion of the serum was filtered six times through qualitative paper and 3 c.c. then injected, intravenously as before. The pig was beautifully anesthetized for a few minutes, but recovered sharply and completely, in a manner very similar to the recovery of those pigs receiving a sublethal dose of the treated serum without filtration. Another portion of the treated and centrifuged serum was placed in a wide mouth test tube and air blown over the surface for two hours. Bubbling air through the serum was impracticable because of the foaming. Injection in 3 c.c. doses produced death, but only varying degrees of stupor in lesser quantities, 1 c.c. being entirely without effect. Again, some normal serum was shaken with one per cent its volume of chloroform for exactly one minute, then allowed to subside for five minutes and the serum carefully pipetted off. Three cubic centimeters of this serum produced an immediate and deep anesthesia lasting for five minutes, when the pig recovered as sharply as it had been prostrated.

All this is preliminary to the object of this paper and only relates the manner in which our attention was temporarily diverted from another line of work to a consideration of certain very obvious phases of Jobling's serotoxin studies. Several objections can be raised at this point to any assumptions, on the basis of the experiments thus far cited, concerning serotoxin. First, rabbit serum is at best but weakly tryptic, and would therefore be unlikely to produce a serotoxin of any remarkable potency. Again, the quantity of chloroform used was too small, according to Jobling, to produce a serotoxin by lipoid removal. Lastly sufficient time was not allowed for the tryptic action to assert itself in full force.

Accordingly various sera were tried and the method of procedure modified to give better conditions for the serotoxin to be formed. In the next experiment rabbit serum was sealed in tubes with chloroform in the proportions of one-tenth volume, one volume, and two volumes of the chloroform to that of the serum. As control tubes, physiological salt solution with the same proportions of chloroform were made up and a tube of the serum with no chloroform. All were placed on the shaking machine at room temperature, and agitated for

the times indicated in the tabulations below, then centrifuged at 8,000 R.P.M. for thirty-five to sixty minutes, and further treated as specified in each protocol.

TABLE II.

Rabbit serum plus one-tenth volume of chloroform, shaken 36 hours at room temperature, centrifuged 35 minutes at 8,000 R.P.M.

FIG.	DOSE.	RESULT.
1	1.5 c.c.	Death in 6 minutes; no shock, some dyspnea, lungs dilated and clot in the heart.
1	1 c.c.	Death in 2 minutes; slight dyspnea, no clot, no dilation of the lungs.
3	1 c.c.	Prostration; recovery sharp and complete.
4	.5 c.c.	No effect.

A portion of the treated and centrifuged serum was aerated for 15 minutes by blowing air through it from a very fine capillary.

5	1 c.c.	No effect.
6	1.5 c.c.	Slight prostration; lived.
7	2 c.c.	Prostration 2 minutes; lived.
8	3 c.c.	Prostration 3 minutes; lived.
*9	1 c.c.	Slight shock; lived.
*10	1.5 c.c.	Marked shock; lived.
*11	3 c.c.	Typical anaphylactic death and autopsy.

*Controls; received serum shaken 36 hours with no chloroform.

TABLE III.

Rabbit serum shaken 36 hours with one volume of chloroform, then centrifuged 35 minutes at 8,000 R.P.M.

FIG.	DOSE.	RESULT.
1	1 c.c.	Prostration; lived.
2	2 c.c.	Deep prostration; recovery gradual and slow.
3	2 c.c.	Death in 2 minutes; atypical, autopsy likewise.

A portion was aerated 45 minutes by blowing air through a fine capillary.

4	2 c.c.	No effect.
*5	2 c.c.	Severe prostration; recovered.
*6	3 c.c.	Death in 2 minutes; atypical death and autopsy.

*Controls; received physiological salt solution treated with two volumes of chloroform, shaken 36 hours, centrifuged 35 minutes at high speed.

In the next experiments two radical modifications were made in the procedure. The centrifugation is continued at the usual high rate for one hour. The aeration was accomplished by placing the serum after treatment and centrifugation, in a wide tube and then directing a strong current of air tangentially against the surface of the serum close to the wall of the tube. The serum by this method was set into violent agitation, but the troublesome foaming was entirely eliminated. The air used was filtered through a long tube filled with glass wool.

TABLE IV.

Rabbit serum plus one-tenth volume chloroform, shaken 18 hours, centrifuged one hour.

FIG.	DOSE.	RESULT.
1	1 c.c.	Stupor; lived.
2	2 c.c.	Deep prostration; recovered.
3	2 c.c.	Instantaneous death; atypical symptoms and autopsy.
4	2.5 c.c.	Death in 1 minute; atypical, autopsy likewise.

A portion was aerated 30 minutes by surface agitation.

5	5 c.c.	No effect.
*6	3 c.c.	No effect.
*7	5 c.c.	No effect.

*Controls; received serum when fresh and without treatment.

TABLE V.

Rabbit serum plus one volume of chloroform, shaken 18 hours, centrifuged one hour.

FIG.	DOSE.	RESULT.
1	1 c.c.	Prostration 2 minutes; recovery sharp and complete.
2	2 c.c.	Death in 2 minutes; atypical.
A portion was aerated 30 minutes by agitation.		
3	2 c.c.	No effect.
4	2 c.c.	No effect.
Another portion was aerated 45 minutes by agitation.		
5	4 c.c.	No effect.
6	5 c.c.	No effect.
*7	3 c.c.	Death in 5 minutes; atypical.
*8	3 c.c.	No effect.
*9	3 c.c.	Slight intoxication; no shock.
#10	5 c.c.	No effect.
#11	5 c.c.	No effect.

*Controls; received serum shaken 18 hours without chloroform.

#Controls; received normal serum standing in cold 18 hours.

TABLE VI.

Physiological salt solution plus one-tenth volume chloroform, shaken 18 hours, centrifuged one hour.

FIG.	DOSE.	RESULT.
1	2 c.c.	Deep prostration; lived.
Aerated 30 minutes by bubbling air through the solution.		
2	5 c.c.	No effect.
*3	5 c.c.	No effect.

*Control; received physiological salt solution.

TABLE VII.

A .5% solution of chloroform in physiological salt solution was shaken 18 hours. Centrifugation was omitted as no chloroform settled out upon standing.

FIG.	DOSE.	RESULT.
1	2 c.c.	Instantaneous death.
Solution was diluted to two volumes with salt solution.		
2	2 c.c.	Prostration and slow death in 5 minutes.
3	1 c.c.	Prostration 1 minute; lived.

We next wished to show that the clarity of the serum was no proof that it was free from chloroform. Rabbit serum was placed with two volumes of chloroform, with occasional shaking at room temperature four days. It was then centrifuged for exactly ten minutes, which was not sufficient centrifugation to make a complete separation of the emulsion, as was evidenced by the cloudiness of the serum. A series of filtrations was then begun with injections intervening to check the progressive partial removal of the residual chloroform. The results are shown in table VIII.

TABLE VIII.

FIG.	WT.	DOSE.	C.C. PER GM. OF WT.	NO. OF FILTRATIONS.	RESULT.
1	350	.5 c.c.	.0014	0	Prostrated 6 minutes; lived.
2	290	.75 c.c.	.0026	2	No effect.
3	220	1.5 c.c.	.0068	4	Atypical death in 19 minutes.
4	275	2.0 c.c.	.0072	7	Prostrated 3 minutes; lived.
An unfiltered portion was aerated 20 minutes by agitation.					
5	225	3.0 c.c.	.013	0	Mild shock; lived.
*6		3.0 c.c.			No effect.
*7		5.0 c.c.			No effect.

*Controls; received fresh untreated serum.

After the fourth filtration some of the filtered serum, which was now crystal clear, was placed in a side arm test tube, tightly corked and the side arm connected to a suction pump giving 24 inches of vacuum. The tube with contents was placed in a bath at forty-five degrees C. for 30 minutes, the pump working all the time. Violent ebullition of the serum began almost immediately and lasted about four minutes. Table IX indicates the results of the injections.

TABLE IX.

FIG.	WT.	DOSE.	C.C. PER GM. OF WT.	RESULTS.
1	215	1 c.c.	.0046	No effect.
2	225	3 c.c.	.013	Shock; lived.
*3	235	3 c.c.	.0127	Light shock; lived.

*Control; received serum which had stood at room temperature 4 days.

At the same time that the serum used in tabulations VIII and IX was placed with the chloroform, another portion was placed with two volumes of ether and with occasional shaking was allowed to stand at room temperature seven days. It was likewise centrifuged ten minutes and the serum removed from underneath the ether by means of a bulb pipette provided with a very fine capillary stem, so as to minimize the quantity of ether which would unavoidably be taken in as the tube penetrated the ether to reach the serum. Table X records the injections and results.

*TABLE X.

FIG.	WT.	DOSE.	C.C. PER GM. OF WT.	RESULTS.
1	245	1 c.c.	.0041	Slight prostration.
2	275	1 c.c.	.0036	Prostration 3 minutes; lived.
3	245	1.5 c.c.	.0061	Prostration four minutes; lived.
The remainder was aerated 30 minutes by agitation.				
4	250	2 c.c.	.008	No effect.
5	285	3 c.c.	.011	No effect.
6	225	4 c.c.	.018	No effect.

*Controls same as in table eight.

In table XI is condensed the last experiment on rabbit serum. This was done in order to make a comparison of the two methods of chloroform removal on the same treated serum.

TABLE XI.

Rabbit serum plus one volume chloroform, shaken 22 hours, centrifuged one hour.

FIG.	WT.	DOSE.	C.C. PER GM. OF WT.	RESULTS.
1	340	.5 c.c.	.0015	Slight stupor.
2	400	1 c.c.	.0025	Death in 4 minutes; atypical autopsy.
A portion was aerated 30 minutes by agitation.				
3	400	2 c.c.	.005	Light shock; lived.
4	285	3 c.c.	.011	No effect.

A portion was placed at 45 degrees C. under a 24 inch vacuum for 5 minutes. Serum boiled vigorously for 2-3 minutes.

5	295	1 c.c.	.0034	No effect.
6	270	2 c.c.	.0074	Prostration 4 minutes; lived.

Still another portion was left at 45 degrees C. for 30 minutes, the vacuum pump acting continuously.

FIG.	WT.	DOSE.	C.C. PFR GM. OF WT.	RESULTS.
7	285	3 c.c.	.011	Light shock; lived.
*8	335	3 c.c.	.009	No effect.
*9	315	5 c.c.	.016	No effect.
#10	290	3 c.c.	.01	No effect.
#11	240	5 c.c.	.021	No effect.

*Controls; received fresh normal serum.

#Controls; received serum shaken 22 hours without chloroform.

The same procedure as has been given thus far was followed quite closely in the work on other sera than rabbit. The data will be tabulated for each serum, and such minor modifications as were made noted.

TABLE XII.

Dog serum plus two volumes of chloroform, shaken 18 hours, centrifuged one hour.

FIG.	DOSE.	RESULT.
1	1 c.c.	Instantaneous atypical death.
Aerated 30 minutes by agitation.		
2	2 c.c.	Shocked; died in 2 hours, typical autopsy.
3	2.5 c.c.	Moderate shock; lived.
4	1 c.c.	Mild shock; died in 12 hours.
*5	3 c.c.	Death in 7 minutes; typical autopsy.
*6	2 c.c.	No shock; died in 15 hours.
*7	2.5 c.c.	Typical shock and death in 6 minutes; autopsy likewise.
*8	2 c.c.	Death in 1½ hours.

*Controls; received fresh normal dog serum.

TABLE XIII.

Dog serum plus one volume of chloroform, shaken 18 hours, centrifuged 1 hour.

FIG.	DOSE.	RESULTS.
1	1 c.c.	Instantaneous death.
Aerated 30 minutes by agitation.		
2	2 c.c.	Violent shock and death in 23 minutes; typical autopsy.
3	2 c.c.	Shock and death in 31 minutes; lungs contracted, other autopsy findings typical.
4	1 c.c.	Mild shock and death in 15 minutes; typical autopsy.
5	2.5 c.c.	Violent immediate shock followed by gradual collapse and death in 43 minutes; no clot, lungs contracted.
*6	2.5 c.c.	Death in 18 hours.
*7	3 c.c.	Death in 2 hours; no shock, typical autopsy.
*8	3.5 c.c.	Typical death in 7 minutes; autopsy likewise.

*Controls; received dog serum shaken 18 hours without chloroform.

TABLE XIV.

Dog serum plus one-tenth volume chloroform, shaken 18 hours, centrifuged one hour.

FIG.	DOSE.	RESULTS.
1	1 c.c.	Instantaneous death.
Aerated 30 minutes by agitation.		
2	2 c.c.	Immediate shock; died in two days.
3	1 c.c.	Light shock; lived.
4	2.5 c.c.	Moderate shock; lived.
5	3 c.c.	Shock; lived.
*6	2.5 c.c.	Shock; lived.
*7	3 c.c.	Shock; and typical death in 39 minutes, autopsy likewise.
*8	3.5 c.c.	Typical death in 5 minutes.

*Controls; received normal dog serum standing in cold for 18 hours.

TABLE XV.

Dog serum plus one volume of chloroform, shaken 19 hours, centrifuged one hour.

C.C. PER GM.				
FIG.	WT.	DOSE.	OF WT.	RESULTS.
1	400	1 c.c.	.0025	Instantaneous death.
2	280	.5 c.c.	.0018	Death in 30 seconds.
Aerated 45 minutes by agitation.				
3	260	3 c.c.	.012	Death in 30 minutes; lungs contracted, no clot, heart in diastole and beating.
4	230	2 c.c.	.0087	Mild immediate shock; died in 14 hours, autopsy same as #3.
5	260	3 c.c.	.012	Death in 25 minutes; autopsy same as #3.
6	175	1 c.c.	.0057	Death in 2½ hours; no shock, autopsy same as #3.
7	270	3.5 c.c.	.013	Death in two hours; autopsy same as #3.
*8	300	3 c.c.	.01	Immediate shock; death in 14 hours.
*9	350	3.5 c.c.	.01	Immediate shock; death in 3 hours.
*10	250	3 c.c.	.012	Shock and death in 6 minutes; autopsy same as #3.
*11	240	3 c.c.	.0125	Immediate shock and death in 23 minutes; autopsy same as #3.
*12	400	5 c.c.	.0125	Immediate shock; death in 2½ hours, autopsy same as #3.
*13	260	3 c.c.	.0115	Immediate shock and death in 7 minutes; autopsy same as #3.
!14	240	3 c.c.	.0125	No effect.
!15	225	3.5 c.c.	.015	No effect.
!16	240	4 c.c.	.017	Immediate shock; death 1½ hours, autopsy typical.
!17	190	3.5 c.c.	.018	No effect.

*Controls; received fresh normal dog serum.

!Controls; received dog serum shaken 19 hours without chloroform.

TABLE XVI.

Guinea-pig serum plus one volume of chloroform, shaken 21 hours, centrifuged one hour.

FIG.	DOSE.	RESULTS.
1	.5 c.c.	One minute prostration.
2	1 c.c.	Deep prostration.
Aerated 30 minutes by agitation.		
3	4 c.c.	No effect.
4	5 c.c.	No effect.
*5	3 c.c.	No effect.
*6	3.5 c.c.	No effect.
#7	5 c.c.	No effect.

*Controls; received homologous serum shaken 21 hours.

#Controls; received homologous serum after standing in cold 21 hours.

TABLE XVII.

Rat serum plus one volume chloroform, shaken 17 hours, centrifuged one hour.

FIG.	DOSE.	RESULTS.
1	1 c.c.	Atypical death in one minute.
Aerated 45 minutes by agitation.		
2	2 c.c.	Shock; lived.
3	3 c.c.	Typical death in 5 minutes.
4	2 c.c.	Shock; lived.
5	2 c.c.	Mild shock; lived.
6	2.5 c.c.	Typical death in 3 minutes.
7	1 c.c.	Severe shock; lived.
*8	3 c.c.	Typical death in 2 minutes.
*9	2 c.c.	Mild shock; lived.
*10	3 c.c.	No effect.
#11	3 c.c.	Typical death in 2 minutes.

*Controls; received fresh pooled rat serum.

#Controls; received normal rat serum shaken 19 hours without chloroform, becoming slightly coagulated.

TABLE XVIII.

Cat serum plus one volume of chloroform, shaken 22 hours, centrifuged one hour.

FIG.	DOSE.	RESULTS.
1	1 c.c.	Instantaneous death.
	Aerated 30 minutes by agitation.	
2	2 c.c.	Slight shock followed by slow collapse and death in one hour.
	Further aerated for 15 minutes by agitation.	
3	2 c.c.	No effect.
4	3 c.c.	No effect.
5	4 c.c.	No effect.
*6	3 c.c.	No effect.
*7	5 c.c.	No effect.
#8	4 c.c.	No effect.
?9	4 c.c.	No effect.

*Controls; received fresh normal cat serum.

#Controls; received serum shaken 22 hours without chloroform.

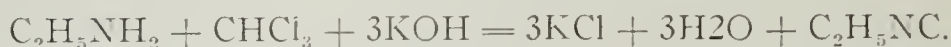
DISCUSSION.

A study of the data given in the preceding tables shows the sera to fall sharply into two groups on the basis of their toxicity either before or after treatment with the chloroform. The first group is composed of the sera of the rabbit, cat, and the homologous serum, guinea-pig. All these when fresh are normally atoxic, and fail after treatment to show the production of anaphylatoxin. The other group is that of rat and dog sera. In this case the question becomes one of the increase of the toxicity by the chloroform treatment, since both are variably normally toxic for guinea-pigs. In table XII, animal 7 shows a typical death and autopsy with an injection of 2.5 c.c. of fresh dog serum. Animal 6 of the same table died in 15 hours, receiving only 2 c.c. of the same serum, but the length of time involved should rule out this and all other deaths occurring more than 30 minutes after the injection. In table XIII, animal 4 showed a typical death in 15 minutes, receiving but one cubic centimeter of the treated serum. The M.L.D. of the untreated serum is observed to be 3 c.c.

With rat serum the M.L.D. was likewise 3 c.c. for the normal serum; after treatment it dropped to 2.5 c.c. Injection of other quantities of both treated and untreated rat and dog sera, produced shocks, but such effects are rather characteristic of both these sera, their toxicity seeming to depend upon some labile conditions that are far from being constant in their action upon injection. Between these extremes there is observed to be considerable common ground to the treated and the untreated rat and dog sera, in which the toxic effects are nearly parallel.

In table VII is shown how small a quantity of chloroform is required to produce death or marked prostration effects when intravenously given to guinea-pigs. This point was tested again and again and the data given is only representative of a large number of experiments made to determine rather closely the M.L.D. of a solution of chloroform. From sera, chloroform is removed only with great difficulty, and since they do not retain chloroform to an equal degree one is never sure when it is completely removed except by trial upon animals. In our work 30 to 45 minutes aeration was found to be required. The dog serum always came from the centrifugation foggy in appearance. In this case the emulsion formed was so fine that centrifugation at 8,000 R.P.M. for

one hour failed to completely remove it. Upon agitation with air for 5 to 10 minutes, or filtration through qualitative paper 4 to 6 times, the serum would become crystal clear, which might easily be assumed to indicate a complete removal of the chloroform. Injection tests running alongside such repeated filtrations, as recorded in table VIII, show clearly that filtration will not remove the chloroform sufficiently, but that it will give marked effects upon injection. Further by applying a very delicate test for chloroform we were able to show that even after the supposedly complete removal by any of the rather strenuous methods employed, there still remained easily detected quantities of chloroform. The method of detection was to make use of the carbylamine reaction,² represented by the equation;



About equal quantities of the serum and alcoholic potash³ are placed in a tube with one drop of aniline. Upon heating the characteristic odor of carbylamine is evolved. One drop of the treated serum before chloroform would give a most pronounced test, and the test never failed when applied to the sera after removal of the chloroform by any of the drastic means employed. How great the influence of this minute quantity of chloroform might be is only a matter for conjecture, however it would seem logical, that since 2 c.c. of a .25 per cent salt solution of chloroform will kill, that much smaller quantities accompanying the serum would not be entirely without effect.

The wide divergency in the character of the deaths of the animals dying from the treated serum as compared to those dying from the untreated serum was most striking. Those animals receiving the first injection of the serum after centrifugation, before any chloroform removal had been attempted, would in nearly every case be dead before the injection could be completed. When the chloroform was but partially removed, a sudden and very typical anesthesia, designated in the tables as prostration, would ensue. The normally toxic sera would give the characteristic jerks of anaphylaxis when untreated, and the same thing would occur after treatment, except that the animal would always be prostrated, making no attempt to gain its feet or to move about. In some instances such animals would never revive, but after a shock of varying intensity, would lie for hours immovable, and the exact time of their death would be uncertain as the typical dyspnea of anaphylactized animals would be lacking.

A very noticeable feature of the effect of the chloroform on the sera was the voluminous precipitate of the serum proteins produced. In this connection, the quantity of chloroform used seemed to exert no influence, those sera treated with but one-tenth their volume of chloroform yielding just as heavy precipitates as when treated with the larger quantities. Also, it made no difference whether the serum was on the machine being shaken constantly, or standing with only an occasional shaking. Time was of but slight consequence, the volume of the precipitate seeming not to increase after the first few minutes of its contact with the chloroform. The precipitates were white or nearly so, sometimes slightly tinged by hemolysis, and were granular to creamy in consistency. These precipitates consisted of the serum proteins. A filtered salt solution responded to the protein color tests, excepting the glyoxylic. A partial

precipitation could be induced by saturation with magnesium sulphate or half saturation with ammonium sulphate. When suspended in water and dialyzed for some time, the dialysate gave the xanthoproteic reaction, showing a water soluble portion. Dilute acids gave only a cloudiness, but strong acids produced marked coagulation, with solution upon excess and heating. The alkaloidal precipitants gave the usual precipitates from the dilute salt solution. It was concluded that the precipitates were the serum albumins and globulins.

It will be observed that in some cases the rabbit and rat sera when shaken without the addition of chloroform became appreciably toxic. In the case of the rat serum a visible agglutination of the serum had taken place, the rabbit serum sometimes showing a slight lack of clarity, but no definite coagulum. Undoubtedly the shaking produced some change in the dispersion of the serum contents that resulted in toxicity.

Keeping in mind the possibilities of error, the rabbit and the dog serum were tested, both before and after treatment with chloroform, for their iodine absorption. These two sera were considered in a measure as being representative of the two groupings mentioned earlier. A measured quantity of the serum was placed in a tube together with some chloroform to act as a solvent, and then a known iodine solution added. Controls were made of tubes without serum. The tubes would then be shaken for 15 minutes and the excess iodine titrated back with sodium thiosulphate. The iodine absorbed was calculated to grams per cubic centimeter of serum. Table XIX gives the results.

TABLE XIX.

Iodine absorption by treated and untreated serum.

SOURCE.	WHOLE SERUM.	TREATED SERUM.
Dog	.0056 gm.	.0048 gm.
Rabbit	.0068 "	.0061 "

It will be recalled that this work was based upon the assumption of anaphylatoxin production by the removal of lipoidal antitryptic substances by chloroform. The iodine absorption would indicate a decrease in the unsaturated bodies of the serum, but not their complete removal. In view of the vigorous treatment employed for their removal, assuming them to be soluble in chloroform, this conclusion is untenable. As an explanation for the disappearance of the iodine, it seems it could be accounted for on the grounds of adsorption by the serum colloids. The lack of distinctness in the end-point, so characteristic of iodine titrations, in this work, would indicate iodine in some rather loose combination with the serum bodies.

In conclusion I wish to express my thanks to Dr. V. C. Vaughan and to Prof. P. H. DeKruif for kindly criticisms and suggestions and to Mr. William German for material assistance in this work.

CONCLUSIONS.

I. The intravenous injection of high dilutions of chloroform may cause sudden death in guinea-pigs.

II. When blood serum has been shaken with chloroform the complete removal of the chloroform is difficult.

III. The toxicity of the serum falls with the completeness of the removal of the chloroform.

IV. Death in guinea-pigs caused by the intravenous injection of serum which has been shaken with chloroform, is often at least, not typical of anaphylactic shock.

V. Serum when shaken without chloroform may cause typical anaphylactic shock with death and typical autopsy findings.

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MICROSCOPIC STUDY OF THE FRESH BILE IN DISEASE OF THE LIVER*

BY H. L. McNEIL, M.D., GALVESTON, TEXAS.

WITH the exception of Bondi,¹ who reports the finding of "Gallenzyylinder" in the fresh bile removed by means of the duodenal tube (after a test meal) from a case of cirrhosis of the liver, we are not aware of any successful observations as to microscopical abnormalities in the bile of such cases. In one case of cholecystitis, also, the same observer noted numerous pus cells in the fresh bile. Petry² and others³ have attempted, unsuccessfully, to obtain information from such studies. All have, however, so far as we are aware, attempted such studies after a test meal of some kind instead of obtaining the pure and fresh secretion undiluted by test meals of water, tea, beef juice, etc.

The series which we have studied consists of thirty cases of disease of the liver. In addition to these, however, about 120 other cases have been studied in a similar manner, these latter cases being either normal or suffering from some disease other than that of the liver. As a result of this work we believe that certain definite and abnormal microscopical changes can be detected in the bile in certain diseases of the liver and its appendages.

As to the technic which we employ, suffice it to say that the duodenal tube is passed upon a perfectly empty stomach, no fluids whatever being allowed for several hours preceding the test (12 hours). Another most important point, which must be observed, is the necessity for an immediate examination of the duodenal contents as soon as removed, since only a few minutes are required for either the complete destruction of the important cellular elements, or for such serious mutilation by the pancreatic ferments as to make them unrecognizable. It is necessary, moreover, to wait until the duodenal contents have become alkaline or neutral in reaction before making the microscopic examination, since the presence of an excess of acid will, by precipitating the bile salts, completely obscure the microscopic picture obtained in an alkaline medium.

*From the University of Texas Medical School.

The microscopic examination is made in the same manner that fresh urine is examined for casts, etc. The fresh contents are placed upon a slide and studied first with a low power lens, then, as soon as a suspicious group of cells is noted, the details are studied with the high power. The oil immersion is not employed. The duodenal contents may be centrifuged. As a rule, however, we omit the centrifuging, since it takes time, contenting ourselves with several examinations of the uncentrifuged specimen.

The appearance of the normal duodenal contents, prepared in this way, is easily described, since practically no cellular elements are noted, except an occasional epithelial cell or a few leucocytes, from the stomach, or a squamous epithelial cell from the mouth or esophagus. Such cells are quite easily recognized by anyone with experience in the microscopic appearance of the gastric contents.

Abnormal microscopic findings in the duodenal contents may be divided, in our experience, into six classes: *First*, bile-stained, granular casts, often suggesting casts of the finer bile passages, occasionally containing a bile-stained cell.* *Second*, bile-stained leucocytes, usually polymorphonuclear, often occurring in groups, and often associated with the third abnormality. *Third*, a round, oval, deeply bile-stained, refractile cell about twice the size of a polymorphonuclear leucocyte, and containing a round, small and often eccentric nucleus. This cell is extremely fragile, disappearing from the field in a very few minutes, being replaced by deeply bile-stained, amorphous and highly refractile masses. Occasionally such cells are found in the form of small casts. *Fourth*, columnar, bile-stained epithelial cells, apparently from the linings of the gall bladder or gall ducts, often occurring in small masses. *Fifth*, the occurrence of large numbers of bacteria, yeasts or protozoa. Normally bacteria do not form a prominent picture in the fresh duodenal contents, although an occasional bacillus or a few cocci may be seen. Yeasts and protozoa are never found under normal conditions. In certain conditions, however, described later, the field may be teeming with animal life. *Sixth*, normally the pure duodenal contents are fluid, containing very little mucus, except that which comes from the stomach. Any marked increase of mucus, especially if associated with the cellular elements already noted, is of significance.

Of eight cases of advanced atrophic cirrhosis of the liver studied in the manner described, we found the first abnormality, amorphous bile-stained casts, in three. The second abnormality, bile-stained pus cells were found in three cases, and the round, refractile cells, deeply bile-stained were found in five cases. Three of these cases subsequently came to autopsy and one to operation, when the diagnosis was confirmed. Two cases of atrophic cirrhosis gave quite negative results on microscopic examination of the bile.

The next most fruitful source of study for us by this method has been four cases of cholecystitis, three of which were acute. In two of the acute cases bile-stained pus cells were found, in one case numerous, in the other case being very few. The round, refractile cell, described as the third abnormality, was found in all three of these cases. In one of these cases innumerable staphylococci were

*"Gallenzyylinder" of Bondi (loc. cit.).

also noted; in another large numbers of motile bacilli formed a prominent part of the picture. In one of these cases, moreover, large numbers of non-bile-stained polymorphonuclear cells were noted.* The one case of chronic cholecystitis, with occlusion of the cystic duct, was quite negative.

The bile from five cases of syphilitic cirrhosis of the liver has not shown as many cellular changes as we might expect. In one case the large, refractile cells, above described, were fairly numerous, associated also with a few pus cells, which were deeply bile-stained. The four other cases were, however, quite negative, except that one case, after repeated examinations, finally showed a very few bile-stained polymorpho-leucocytes. None could be found by similar methods in the other three, however. The diagnosis in all of these cases was made from the clinical signs, the positive Wassermann reaction and the therapeutic test.

Four cases giving alcoholic histories, showing large and tender livers, and having negative Wassermann reactions, were studied. Only one of these cases showed any important cellular changes. In this case abnormalities one, two, three and four were noted.

Three cases of tropical liver abscess, studied by this method were quite negative.

One case of carcinoma of the liver was negative.

Four cases of chronic passive congestion of the liver were negative, except for the presence of a few columnar epithelial cells in one case.

One case of acute yellow atrophy of the liver, which ended fatally, was interesting from the fact that bile-stained pus cells were numerous, and were associated with innumerable bacteria (chiefly the colon bacillus, but containing a few cocci).

Two cases, finally, which we were unable to classify, were of interest. The first was a man of fifty, complaining of pain in the right upper abdomen. Physical examination negative except a liver generally enlarged and slightly tender. Wassermann negative, previous history negative. We found large numbers of trichomonas intestinalis in this case, without any cellular changes, however. The gastric contents were also negative microscopically, and contained no protozoa.

The other case, a man of fifty-five, giving a negative alcoholic history and having a negative Wassermann, was admitted to the wards complaining of abdominal pain and tenderness. The liver was large, hard and tender. Physical examination otherwise negative. Microscopic examination of the duodenal contents showed innumerable lamblia intestinalis. These protozoa were so numerous, in fact, that everything else in the field was obscured by them. A large number of pus cells were found in this case.

In our series of cases not suggesting disease of the liver, being either normal or complaining of some form of gastric disturbance, or suffering from other diseases, such as typhoid fever, arthritis, malaria, uncinariasis, cardiac disease, nephritis, toxic vomiting of pregnancy, etc., consisting of some 120 cases, we have noted an occasional abnormality, as follows: In three cases of chronic gastritis, all three of whom gave alcoholic histories, the large, yellow, refractile

*Duodenitis.

cells, above described, were found in greater or less number. In one of these cases, moreover, a case coming to autopsy, fortunately, a few bile-stained pus cells were noted. At autopsy no serious lesion of the liver was found, a rather extensive cloudy swelling being practically the only lesion. One case of hypersecretion and hyperacidity, complaining of the usual pyrosis and gastric distress showed a few of the same round cells in the duodenal contents. In two cases of subacute gastritis, both non-alcoholic, a few of the round, refractile cells were found. With these few exceptions, out of some 120 selected cases, complaining of all forms of gastric disturbances, no abnormalities, such as we have described, have been found.

Finally, we believe that the following conclusions as to the value of the microscopic examination of the fresh duodenal contents may be drawn:

First: In certain diseases of the liver, particularly in atrophic cirrhosis, definite abnormal cellular elements are found in the fresh bile in a certain percentage of cases. It is probable that these cells may be of diagnostic importance in this disease.

Second: Such cellular changes may be of diagnostic importance in certain cases of acute cholecystitis.

Third: Abnormalities in the fresh bile are rarely found in other forms of hepatic disease, such as syphilis of the liver, chronic passive congestion of the liver, abscess and carcinoma of the liver.

Fourth: We believe that such examinations as we have described offer the only satisfactory means of studying inflammations of the duodenum (duodenitis) satisfactorily.

Fifth: In order to obtain satisfactory microscopic pictures, it is absolutely necessary that the bile be examined *immediately* after removal, that it be alkaline or neutral in reaction, and unmixed with food or liquids.

DIAGNOSIS.		GROSS APPEARANCE.	MICROSCOPIC APPEARANCE.
1.	Atrophic Cirrhosis	Turbid Bile +++	Numerous bile-stained pus cells. Hepatic ? cells
2.	" "	Turbid Bile ++	"Gallenzyylinder" bile-stained pus cells ++
3.	" "	Clear. Bile +	Few Hepatic ? cells
4.	" "	Turbid Bile ++	Hepatic ? cells. Bile-stained pus cells (few). "Gallenzyylinder."
5.	" "	Clear Bile ++	Negative
6.	" "	Clear Bile +	"
7.	" "	Clear Bile +++	"Gallenzyylinder." Hepatic ? cells
8.	" "	Turbid Bile +++	Hepatic ? cells
9.	Cholecystitis (acute)	Turbid. Bile ++ Mucus ++	Few bile-stained pus cells. Bile-stained cylindrical epithelial cells.

DIAGNOSIS.		GROSS APPEARANCE.	MICROSCOPIC APPEARANCE.
10.	"	Clear. Bile + Mucus +	Very few bile-stained pus cells
11.	" (typhoid)	Clear. Bile Scanty Mucus +	Bile-stained pus cells fairly numerous. Hepatic ? cells B. Typhosus.
12.	Cholecystitis (chronic) Cystocele	Bile + Clear	Negative
13.	Syphilitic Cirrhosis	Bile + Clear	Hepatic ? cells ++ Few bile-stained pus cells
14.	"	Bile scanty Clear	Few bile-stained pus cells after three trials
15.	"	Bile + Clear	Negative
16.	"	Bile + Clear	"
17.	Syphilitic Cirrhosis	Bile + Clear	Negative
18.	Large tender liver Alcoholic history	Bile ++ Turbid	"Gallenzyylinder." Bile-stained pus cells. Hepatic ? cells.
19.	"	Bile +++ Turbid	Negative
20.	"	Bile +	"
21.	"	Bile +	"
22.	Hepatic Abscess	Clear Bile scanty	"
23.	" "	" "	"
24.	" "	" "	"
25.	Carcinoma Liver	" "	"
26.	Chronic Passive Congestion of Liver	" "	Cylindrical epithelial cells
27.	"	" "	Negative
28.	"	" "	"
29.	"	" "	"
30.	Acute Yellow Atrophy of Liver	Bile ++ Clear	Numerous bacteria (B. Coli and Gram-positive cocci) Pus cells ++
31.	Large tender Liver	Bile ++ Clear	Numerous trichomonds intestinalis
32.	" " "	Bile ++ Clear	Pus cells ++ Innumerable Lamblia intestinalis

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LABORATORY METHODS

Newer Laboratory Methods for the Early Diagnosis of Pulmonary Tuberculosis

BY MORRIS H. KAHN, M.D., NEW YORK CITY.

IN the past decade quite a number of laboratory methods have been suggested for the early diagnosis of pulmonary tuberculosis. The various tests concern themselves with the sputum, the urine, the feces and the blood. Whereas bacteriological examination of the sputum is of absolute diagnostic importance, the other laboratory procedures have given rise to much commentation and dispute and are as yet only of relative value.

ALBUMIN IN THE SPUTUM.

Stimulus was given by Roger,¹ in 1909, to the question of albumin in the sputum of tuberculous patients, and it became the subject of considerable study. The occurrence of albumin in the sputum was mentioned by a few authors since 1855 as present in pneumonia and pulmonary edema,² but it has been shown that in these conditions extravasations of blood constitute the chief source of the albumin.³

The test is made as follows: Ten cubic centimeters of sputum are diluted with an equal amount of water. To this is added one cubic centimeter of glacial acetic acid to precipitate the mucin, and the mixture is filtered through fine filter paper. The filtrate shows albumin by boiling or by the nitric acid test.

The albumin has been supposed to indicate that the sputum is not a mere superficial secretion, but comes from some deep-seated inflammatory process.⁴ Most of the recent investigators have asserted the belief that a negative test for albumin in the sputum contraindicated the existence of tuberculosis,⁵ while a positive test rendered the case only suspicious, since a positive test may also be obtained in pneumonia, pulmonary edema, and heart disease with pulmonary congestion. It was later found that albumin was absent in fibroid tuberculosis.⁶

Some observers felt that an increase or decrease of albumin content of a tuberculous sputum points to aggravation or improvement of the pulmonary lesion, thus placing a prognostic significance to the findings. But Lockwood, in a very recent work,⁸ examined the sputum of thirty cases of asthma, emphysema and bronchitis which had no evidence of tuberculosis. He used a one per cent solution of phosphotungstic acid, in alcohol for precipitation of the albumin. He found albumin in the sputum of all cases examined, tuberculous and nontuberculous. While the average quantity of albumin was found to be higher in the tuberculous cases, there was such a wide variation in the quantity in both the tuberculous and nontuberculous that this could not be used as a diagnostic aid. In the tuberculous patients, the amount seemed to bear no

relation to the stage, duration or activity of the disease process. Yet one deduction seems feasible from the results; i.e., the absence of albumin in the sputum may be deemed as confirmatory proof of the absence of tuberculosis.

FERMENTS IN THE SPUTUM.

The occurrence of ferments in the sputum for diagnosis attracted little study, though observations were made on the subject by Stolnikoff⁹ and Escherich¹⁰ about forty years ago. In the examination of tuberculous sputa by Eiselt¹¹ a proteolytic ferment, chiefly trypsin was found; during febrile periods the trypsin disappeared and was replaced by antitrypsin. No lipolytic ferments were found, but albumoses and peptones were present, probably from the occult bleeding which may have occurred. The ferment action, according to the author, was in inverse proportion to the content of coagulable albumin, and in direct proportion to the amount of albumoses and amino-acids in the sputum.

SALICYLIC ACID TEST.

Falk and Tedesco reported a test in 1909¹² which promised to differentiate disease processes limited to the bronchial mucous membrane from those which had extended to the lungs. The principle of the test was based on the assertion that salicylic acid when present in the blood, appears in inflammatory exudates from the lungs, but is not excreted from the bronchial mucosa. The patient is given thirty grains of salicylic acid and the sputum is collected for twelve hours. The sputum is then tested for salicylic acid by a solution of ferric chloride. A violet color indicates a positive reaction.

GLYCOGEN TEST.

Another observation that has been made on the sputum in pulmonary tuberculosis is the increased proportion of glycogen, reported by Moscati,¹³ and confirmed by Pozzilli¹⁴ and others. Moscati concluded from his studies of many cases, that glycogen always exists in increased quantities in tuberculous sputum, and that it is always increased with destructive lung processes. It forms 2 or 3 per cent in the advanced stages of the disease. Pozzilli tabulated the findings in twenty-two cases of various affections of the air passages. In one case of advanced pulmonary tuberculosis, glycogen formed 3.75 per cent of the sputum, and was never below 2.5 per cent in other advanced cases. In earlier stages of the disease, it ranged from 0.15 to 0.6 per cent. It was not demonstrable in simple bronchial catarrh or in acute bronchitis. In pneumonia, it ranged from 0.05 to 0.06 per cent. The highest proportion detected in any nontuberculous affection, 0.25 per cent, was in a case of putrid bronchitis. The proportion of glycogen may thus prove a valuable aid for differentiation of tuberculosis and for prognosis. The loss of glycogen must be one factor in the debility of consumptives and may also throw light on the peculiar susceptibility of diabetics to tuberculosis.

The glycogen can be demonstrated by the iodine test or by Pflüger's technic. For the former the sputum is rendered slightly alkaline with sodium carbonate, and then heated for an hour to allow the glycogen to dissolve out into the water. The fluid is then filtered and boiled down in the water bath. An

iodo-iodide solution (0.25 parts of iodine; 0.5 part of potassium iodide and 100 parts of water) is poured on the filtrate in a test tube held very slanting. A mahogany ring forms at the junction of the two fluids in the presence of even very small proportions of glycogen.

CYTOLOGICAL STUDIES.

Cytological studies of the sputum have been found to be quite typical in pulmonary tuberculosis. Attention was first called to an increase of a cellular element in the sputum as an early sign of tuberculosis by Wolff-Eisner in 1907.¹⁵ He found an increase in small lymphocytes in early tuberculosis, anywhere from 33 to 90 per cent. The pathologic significance of lymphocytes in the sputum is difficult to explain, but it has probably to do with the relationship of the toxins of the tubercle bacillus to the emigration of leucocytes. A high lymphocytic content of the sputum is in itself strong presumptive evidence of tuberculosis. While conversely, a high polynuclear content speaks against it, except in cases of mixed infection, where, of course, tubercle bacilli are as a rule present in large numbers.¹⁶

Recent studies on the value of the test have confirmed the belief that a sputum lymphocytosis showing under the microscope 50 per cent or more of lymphocytes speaks strongly for the presence of pulmonary tuberculosis. It would seem desirable that a careful cytological examination should be made of every sputum that is negative for tubercle bacilli. If a positive albumin finding is present and a preponderance of lymphocytes, we may be justified in assuming that the source of this expectoration is a tuberculous focus in the lungs.¹⁷

UROCHROMOGEN TEST.

The chemical changes occurring in the urine during tuberculosis have been given considerable study, frequently with contradictory findings. The disparities among the results of different authors are probably chiefly due to the fact that it is difficult to select for such studies a group of tuberculous individuals wholly free from various conditions that influence the urinary output and thus complicate the problem.

A number of investigators, following Weiss, who published his findings in 1910,¹⁸ have reported marked increase in urochromogen in the urines of tuberculous individuals. They have claimed that the amount of urochromogen output increased with the progress of the disease and that in general the amount of urochromogen suggests the degree of tissue destruction in the body.¹⁹ In a number of cases reported, the quantity of urochromogen ran parallel with the other clinical symptoms of the disease, and always became negative in cases which could be considered clinically cured.²⁰ The occurrence of excess of urochromogen has therefore been advocated as of diagnostic as well as prognostic value in tuberculosis.²¹

The test is simple. Five cubic centimeters of urine are diluted with ten cubic centimeters of water. A few drops of a dilute (1-1000) permanganate solution are added. In the presence of excess of urochromogen, a deep yellow color appears. The test seems from its very nature, not specific for tubercu-

losis. The reading is sometimes made difficult by the deep yellow color of certain specimens of urine, and also because a number of normal urines become more yellowish after addition of permanganate. It sometimes occurs in normal urine and is quite constant in typhoid.

The original Ehrlich's diazo reaction has also been used in the diagnosis of chronic tuberculosis. It depends upon the formation of some unidentified coloring matter produced by the combination of some undiscovered substance in the urine with a diazotized aniline.²² Usually a diazotized sulfanilic acid solution with a solution of sodium nitrate is added to an equal volume of urine. A red color forms on the addition of ammonia.

According to Weiss, the normal coloring matter of the urine is derived from a chromogen, which in its properties is similar to the proteic acids which are present in the urine. This is the substance he holds responsible for the Ehrlich's diazo reaction. When this substance is oxidized, either by dilute solution of potassium permanganate or by iodine and potassium thiosulphate it is changed to the true urochrome of the urine.

In 1914 comparative studies were made of urinary findings in two hundred cases.²³ Of these, one hundred were clinically tuberculous and one hundred clinically nontuberculous. Of the former, only forty-six or less than 50 per cent gave a positive Weiss and a positive diazo reaction, and forty-two were negative for both tests. Of the one hundred nontuberculous cases (excluding typhoid) thirteen urines were positive to both tests and seventy-eight urines negative to both. The authors reached the following conclusions:

The Weiss and diazo tests in tuberculosis are not constant, as there are various other pathological conditions in which these tests are positive. The urinary findings are not sufficiently frequent in tuberculosis to be of special diagnostic value, even when other possible complications giving rise to positive tests can be excluded.

These conclusions were corroborated in a more recent study by Bronfenbrenner on tuberculous cases which the author had under observation in his clinic.

TUBERCLE BACILLI IN STOOL.

A few years ago Meyer Solis-Cohen reported six cases in which tubercle bacilli were found in the feces before they were discovered in the sputum.²⁴ In several of the cases at the time, there were no symptoms or signs of intestinal tuberculosis. He therefore suggested routine examination of the feces for tubercle bacilli in cases of pulmonary or general tuberculosis, believing that frequently tubercle bacilli will be found in cases in which the sputum is negative or not obtainable. The importance of this test is obviously doubtful.

ARNETH CLASSIFICATION OF LEUCOCYTES.

Studies of the blood have evolved certain refinements in the differential counting of the leucocytes which may serve to assist in the diagnosis of early pulmonary tuberculosis.

Arneth classified the neutrophile polymorphonuclear leucocytes according to their nuclear division. He considered that certain polymorphonuclear cells, those with one or two nuclei are less resistant and less able to combat infec-

tion than those with more nuclear lobes.²⁵ He found from a study of the blood of fifteen healthy men that the normal picture was as follows,

I	II	III	IV	V
5%	35%	41%	17%	2%

giving an index of 40, counting the sum of classes I and II as the index. He also found that the number of lobes was independent of the total number of leucocytes. Minor and Ringer found a slightly different normal from a study of the blood of ten healthy men,²⁶

I	II	III	IV	V
2.5	18.2	55.6	18.6	5.1

giving an index of 20.7 as the normal. Bushnell and Trueholtz take as their normal index the sum of I plus II plus half of III which would give according to Arneth 60 as the normal and according to Minor 48.5.

In tuberculosis Arneth found that there was an increase of those with single or two lobes and a decrease of III, IV and V. Thus in severe cases, counts were obtained in which there were 36 per cent of I, 56 per cent of II, 8 per cent of III and zero of IV and V. The occurrence of such a deviation to the left, as it is called, was confirmed by Klebs,²⁷ Bushnell and Trueholtz²⁸ and by Minor and Ringer.

The cause of this phenomena is unexplained, but the degree of this deviation from the normal appears to be roughly proportionate to the severity of the infection and may be taken as an estimate of the amount of toxemia. According to some authors such changes in the neutrophiles in tuberculosis offer a distinct aid in prognosis, and serve as a guide to treatment.²⁹

We have made a study of the "Arneth" blood-picture in twenty-seven cases attending the tuberculosis clinics of the New York Health Department. The patients were in various stages of the disease. All showed tubercle bacilli in the sputum at the time of the blood examination.

Arneth used Ehrlich triacid stain. From his work, one gains the impression that he preferred a stain which would not stain the nucleus sharply in order that the threads connecting its subdivisions might not become visible. Bushnell used Delafield's hematoxylin and 0.5 per cent alcoholic solution of eosin, staining first with eosin, washing and then staining with hematoxylin. We used Jenner stain, which we found to give good nuclear differentiation.

To obtain percentages of the different types of cell, we first counted 100 white blood cells, giving directly the percentage of the various leucocytes. Then we counted the neutrophiles alone until we had classified 100 into their Arneth divisions, thus obtaining the percentages of the different classes below. In classifying the neutrophiles, it is at times difficult to say whether the nucleus has two or more lobes. We therefore adhered to the rule that if there is any band of nuclear tissue, except a thin chromatin filament connecting the different parts of a nucleus, the nucleus cannot, for the purposes of the count, be said to be divided. (See Table I.)

The results in our cases agree closely with all other workers.³⁰ In the

more favorable cases, the number of neutrophilic cells of class I and II diminish, while in the severer cases, these cells increase in number. It is difficult to explain why Solis-Cohen and Strickler³¹ obtained results directly opposed to the findings of all other workers, but there is one error obvious in their calculations. While considering their normal index as the combined percentage of neutrophilic cells with one and two nuclei, they make comparison of their index with that of Minor and Ringer and that of Webb and Williams who include half of III in their index count. In our tables, the latter index was calculated.

TABLE I.

STAGE.	I.	II.	III.	IV.	V.	POLYS.	LYMPH.	L. M.	EOS.	BAS.	INDEX.
I including non-toxic cases.	6	37	44	11	2	66	26	8			65
	15	40	35	9	1	50	37	11	1	1	72
	25	49	19	6	1	45	46	2	5	2	82
	6	27	47	16	4	56	36	4	4		56
	13	46	35	6		67	27	4	1	1	76
	2	52	36	12		73	25	2			72
	11	49	32	7	1	79	19	1	1		76
II including cases with slight fever.	19	57	24			54	37	6	3		88
	34	36	21	9		64	35		1		80
	29	33	30	7	1	56	32	6	6		77
	22	40	33	4	1	71	27		2		78
	14	48	31	7		66	24	9	1		77
	45	30	24		1	57	34	7	2		87
	12	46	38	3	1	66	27	4	3		77
	12	49	34	5		59	37		3	1	78
	9	55	31	5		85	15				79
	15	40	32	7	6	56	27	16	1		81
	24	48	21	7		78	18	3	1		82
	30	40	27	3		58	34	4	3	1	83
III including cases with high fever, night sweats and marked toxemia.	58	29	12	1		78	20		2		93
	60	32	7	1		56	39	2	2	1	95
	49	39	10	2		61	23	14	2		93
	36	51	12	1		78	17	2	1	2	93
	50	40	8	2		54	33	11	1	1	94
	17	47	26	9	1	72	22	3	3		90
	41	42	12	5		74	15	11			89
	28	54	16	2		74	17	7	1	1	90

BACTERIOLOGIC STUDIES OF THE BLOOD.

Some authors claim to have demonstrated tubercle bacilli regularly by a direct microscopic examination of the blood of tuberculous patients,³² and frequently also by the inoculation of such blood into guinea-pigs and rabbits.³³ Other laboratory workers have failed absolutely to obtain such results.

Now, with regard to the microscopic examinations, there are so many sources of error, such as the presence of acid-fast organisms resembling tubercle bacilli in distilled and tap water and certain saprophytic bacteria as well, the close resemblance of the stroma of the erythrocytes, crystals of lecithin and cholesterin and of flakes of fibrin, that positive reports may be attributed to these factors. The method has proven of no value.

With regard to the inoculation of guinea-pigs with the blood from tuberculous patients, it has been shown that ten tubercle bacilli suffice to inoculate a

guinea-pig successfully³⁴ and some think that this can be accomplished by a single tubercle bacillus introduced intraperitoneally.³⁵ The most recent experiments have shown the test on tuberculous patients to be negative. Blood from forty-seven advanced cases of tuberculosis was injected intraperitoneally into guinea-pigs. Only one of the guinea-pigs who received blood from a patient who had previously had a tuberculin injection, developed tuberculosis. (Kessel, 1915.³⁶) All of the other injections were negative. This method of diagnosis must therefore also be discarded.

Attempts have been made to apply the agglutinin reaction to the diagnosis of tuberculosis. Arloing and Courmont³⁷ used a homogeneous culture of tubercle bacilli transplanted from potato to broth. The authors claimed significant results for the reaction. Kinghorn and Twichell³⁸ repeated the studies by the same method. Clear fresh human serum was used, and was obtained either from the lobe of the ear or from a vein in the arm. With each serum, mixtures of different strengths were prepared, one part of serum to five, ten, fifteen, etc., parts of emulsion of broth culture of tubercle bacilli. When agglutination took place in a dilution of one to five within five hours the reaction was considered positive. The authors obtained 84 per cent positive reactions with the sera tested from healthy persons, and 87 per cent positive from tuberculous sera. There is therefore little, if any, difference between the agglutinating power of healthy and of tuberculous sera.

The precipitin and meiotagmin reactions, and lately the biochemical test of Abderhalden have also been studied for the diagnosis of tuberculosis, but the results were of scientific interest only.

COMPLEMENT FIXATION IN TUBERCULOSIS.

Following the demonstration of the value of the Wassermann reaction for the diagnosis of syphilis in 1906³⁹ many attempts have been made to adapt serologic methods to the diagnosis of tuberculosis.

The method of testing, is based on the phenomenon of Bordet and Gengou,⁴⁰ or fixation of complement, of which the Wassermann test is an atypical example. An antigen, such as the toxin of tubercle bacilli, when put in the presence of its specific antibody, which is supposed to circulate in the blood of tuberculous patients, will unite together, through the intervention of a third thermolabile substance which exists in all serums and is called complement. If one now puts into this triangular mixture of antigen-complement-antibody, a second antigen, say red blood cells and an antibody to these blood cells which will lase them, the red blood cells will not be laked if the complement which is also necessary here, has been used up in the first reaction.

The main reason why complement fixation in tuberculosis has failed seems to have been the lack of a suitable antigen. This gap seems at last to have been filled through the discovery of Besredka of a new method of cultivating tubercle bacilli.⁴¹ With Besredka's method using a mixture of bouillon, egg-yolk, and egg-white, a luxuriant growth occurs within several days. The germs cultured in this way show biologic properties distinctly different from those observed heretofore, one of the most striking being the rapidity of their growth, which almost equals that of streptococcus.⁴² To prepare the antigen, this culture is

heated and deprived of its cellular elements by filtration. The filtrate is titrated for anticomplementary or hemolytic properties, and then used as antigen. Craig⁴² prepared his antigen similarly, using several strains of tubercle bacilli of the human variety grown in alkaline broth to which was added one dram each of egg-white and egg-yolk to 250 c.c.

The author has had the privilege of examining in collaboration with Bronfenbrenner, the comparative value of the Weiss test for neutral sulphur, the Ehrlich's aldehyde test and the Besredka serum test. Fifty-nine cases attending the tuberculosis clinics of this city were studied. The results, which will be published in detail elsewhere in a more comprehensive paper,⁴⁴ may be summarized as follows: The serum test was positive in about 92 per cent of the cases; while the Weiss test was positive in about 13 per cent of the cases, and the diazo test in about 8 per cent.

In first stage cases, the serum test was positive in 84 per cent, while the Weiss test was positive in 4 per cent; in second stage cases the serum test was positive in 94 per cent and the Weiss test was positive in 9 per cent, whereas in third stage cases, the serum test was positive in only 15 per cent whereas the positive urochromogen test rose to 31 per cent.

It is therefore to be concluded that the urinary findings do not go parallel with the serum findings. On the contrary, the latter were very high in the primary and secondary stages of pulmonary tuberculosis and fell very markedly in the tertiary stage of the disease, while the urinary findings seemed to show a very marked increase as the disease progressed.

Tuberculin of Besredka seemed to give the best results in diagnosis by complement deviation test. Even though the test was positive in a certain number of clinically nontuberculous cases, the reaction seemed to be specific. At least in 87 per cent of such cases, fixation was also obtained by one or more tuberculins other than that of Besredka, and such findings can well be attributed to the fact that in its earlier stages, the tuberculous process does not give symptoms sufficient for clinical diagnosis. The increase in the incidence of the Weiss reaction, together with the corresponding inverse frequency of occurrence of the serum test is quite constant in advanced stages of the disease, and may be of prognostic value, the former indicating tissue destruction, and the latter showing the lack of reacting power on the part of the body.

Lastly by a very simple method of preparing tuberculosis antigen by triturating tubercle bacilli in a mortar in salt solution, H. R. Miller⁴⁵ claims to have obtained 100 per cent positive results by complement fixation in positive cases of tuberculosis, and 100 per cent negative results in nontuberculous or clinically cured cases. The results are extremely interesting and require further confirmation.

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The Dissociation Curve as an Index to the Hydrogen-ion Concentration of Blood*

By R. W. SCOTT, M.D., CLEVELAND, OHIO.

IN the study of clinical cases of dyspnea, often referred to as renal or cardiac asthma, more and more attention is being paid to the reaction of the blood. Now, the reaction, or better the hydrogen-ion concentration of the blood, may seem rather remote from the activity of the respiratory center, but consideration of the following physiological facts will show that indeed a very intimate relationship exists.

The cells of the organism have been so sensitized to their environment that, in order to carry on their normal metabolic activity, they require a circulating nutrient medium whose hydrogen-ion concentration remains practically constant. Hence the reaction of the blood and body fluids stands as one of the most constant facts in the whole organism. Since cellular activity demands this constancy of reaction, there must be some mechanism whose function it is to respond to slight alterations, and thereby rapidly restore the blood to its normal reaction. Such a mechanism is the respiratory center.

During metabolic activity there are added to the blood stream certain acid products—the so-called “fixed acids,” which are eliminated slowly by the kidney, and a volatile acid CO_2 , eliminated rapidly through the lungs. Obviously it is this latter mechanism that has to do with the rapid restoration of the blood to its normal reaction. When the blood becomes more acid, either from an increased production or a failure of elimination of these acid products, the respiratory center responds and attempts to reduce the acidity by increasing pulmonary ventilation. This increases the elimination of CO_2 and suffices to reduce the blood to its normal condition, e.g., in physiological exercise, where the acidity is largely due to an increased production of carbon dioxide. In certain pathological cases, however, where the acidity is due to the presence of nonvolatile acids, this mechanism does not suffice and the patient remains dyspneic.

It is seen therefore that in the behavior of the respiratory center there exists the most delicate indicator in the body for the detection of slight alterations in the reaction of the blood. In fact physiological experimentation shows that pulmonary ventilation is increased over 100 per cent by an increase in the hydrogen-ion concentration of the blood which is smaller than can be detected by any known laboratory method.

Next to the respiratory center in order of sensitivity to changes of hydrogen-ion concentration stands the chief respiratory agency of the blood, namely, hemoglobin. As pointed out by Barcroft and others, the affinity of hemoglobin for oxygen bears a constant and definite relation to the hydrogen-ion concentration of the serum. This affinity can be ascertained by exposing blood at body temperature to an atmosphere containing an accurately known pressure of oxygen. The blood is then examined in Barcroft's differential manometer, in order to find what percentage of the O_2 which it ought to absorb at a given O_2

*From the Physiological Laboratory, Western Reserve University Medical School.

pressure has actually been absorbed (the so-called percentage oxygen saturation). The lower the percentage saturation, other conditions being unchanged, the higher the hydrogen-ion concentration of the blood.

To determine the oxygen saturation, the following method, devised by Barcroft¹ and slightly modified by Kato,² is the most useful for the study of clinical cases.

Description of Method.—One to two-tenths c.c. of defibrinated blood are drawn into the stem of a small serological pipette and allowed to flow into the bulbous portion. The neck is connected to the delivery tube of a Berzelius gas holder by means of a small piece of thick-walled rubber tubing. It has been found convenient to interpose a small wash bottle to gauge the rate of flow of the gas. The stem of the pipette is introduced into a three-inch piece of glass tubing, which is clamped to a wood block in a water bath—care being taken to have the bulb containing the blood well covered with water. The bath is kept at 38° C. by a thermo-regulator.

The pipette is kept in constant rotation either by hand or better by means of a small electric motor equipped with an eccentric. A cord is passed under the pipette just below the bulb. (A small bit of rubber tubing placed at this point will offer sufficient resistance to insure good rotation.) One end of the cord is attached to the eccentric, the other to an ordinary rubber band tied to an iron stand.

To prevent the entry of water into the tip when the pipette is immersed in the bath, we have used a suitable bit of rubber tubing over the end of the stem. The free end is plugged with a glass bead. Just before the introduction of the gas the tubing is elevated above the surface of the water and the plug removed. Again when the pipette is removed from the bath the bead is inserted to prevent the ingress of air due to contraction from cooling.

Rotation is continued for ten minutes, during which time a slow but continuous stream of gas is allowed to flow over the blood. The pipette is now removed from the bath. A little gas from the holder is now introduced in order to establish a slight positive pressure within the bulb, so that when the tubing over the end is removed there is no ingress of air. The pipette is held upright and the blood forced down into the stem by compressing the rubber tubing connected to the neck, this tube having previously been clamped and disconnected from the gas holder. The blood is delivered under dilute ammonia water contained in the gas bottle of the Barcroft differential manometer and its percentage saturation determined as described by Barcroft.

Normal blood gives a percentage saturation of 69 to 74 per cent at 17 mm. O₂ pressure in the absence of CO₂, that is to say, when human blood is exposed to an atmosphere containing 17 mm. O₂ at body temperature, 75 per cent of the hemoglobin is oxyhemoglobin and 25 per cent reduced hemoglobin. If this figure is lowered (e.g., to 50 per cent, so that 50 per cent of the sample is oxyhemoglobin and 50 per cent reduced hemoglobin), it indicates a certain increase in the hydrogen-ion concentration of the blood.

¹Barcroft: Respiratory Function of the Blood.

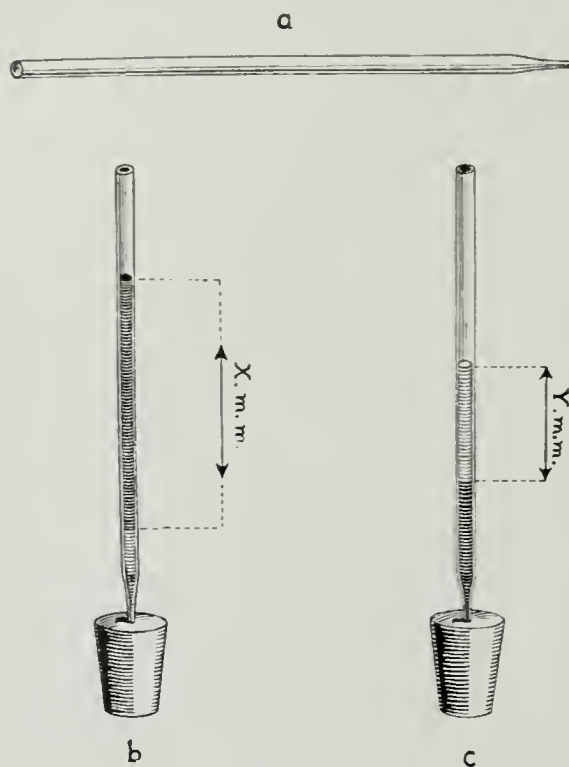
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A Simplified Hematocrit and A Method For Determining Variations in Blood Volume*

BY ALBERT A. EPSTEIN, M.D., NEW YORK CITY, N. Y.

THE apparatus (see diagram, Fig. a) consists of small glass tubes made from selected glass tubing of uniform bore (0.5 to 1 mm. in diameter and 6 to 8 cm. long) drawn out at one end into a fine capillary. The blood is obtained from the finger tip (lobe of the ear or vein) in the usual manner. Clotting of the blood is prevented by means of finely powdered hirudin.

Just before drawing the blood, the wide end of the tube is dipped into the powdered hirudin and withdrawn. A few granules of the substance (usually sufficient to prevent clotting of the blood) adhere to it. After the finger is pricked and the blood oozes out easily and without pressure, the end of the tube containing the hirudin is brought in contact with the blood which flows readily



Formulae for computing percentage volume of plasma and blood cells.

$$(1) \text{ Per cent of plasma} = \frac{y}{x} \times 100.$$

$$(2) \text{ Per cent of blood cells} = \frac{x-y}{x} \times 100.$$

into the tube. The tube is half filled with blood, and is then tilted two or three times to permit the hirudin to mix with the blood. Holding the tube slightly inclined with the capillary end downward, the blood is allowed to run down to about one centimeter from the capillary end, which is then sealed in a flame. A minute test tube is thus formed. The column of blood (which remains separated from the bottom of the tube by a cushion of air) is then measured by means of a millimeter scale and its length carefully determined. The sealed end of the tube is then inserted into a cork (see diagram, Fig. b) (which has a small hole

*From the Pathological Laboratory of the Mount Sinai Hospital, New York City, N. Y.

in it) and centrifuged for ten minutes at the rate of about 2,500 to 2,600 revolutions per minute. The cells are completely sedimented.* The column of supernatant plasma (see diagram, Fig. c), which is clear and sharply separated from the cells, is measured by means of the millimeter scale and its length determined. The difference between the column of plasma and the original column of blood represents the volume occupied by the blood cells. The value of both plasma and cells may be computed in terms of per cent by dividing the figure obtained for each of them by that of the original column of blood, and the quotient multiplied by 100.

Example:

$$\frac{\text{Column of blood cells in millimeters} \times 100}{\text{Original column of blood in millimeters}} = \begin{array}{l} \text{Volume of blood cells} \\ \text{in the blood} \\ \text{in per cent.} \end{array}$$

$$\frac{\text{Column of plasma in millimeters} \times 100}{\text{Original column of blood in millimeters}} = \begin{array}{l} \text{Volume of plasma} \\ \text{in the blood} \\ \text{in per cent.} \end{array}$$

A different tube of the type described above is used for each determination of the relative content of cells and plasma in the blood. The accuracy of this method of measuring the blood cells and plasma is confirmed by the fact that in duplicate and triplicate determinations the results agree, although the amount of blood used in the several determinations need not be exactly the same.

Changes in the blood volume can be ascertained by means of repeated determinations of the relative amounts of plasma and cells present in the blood. It can be safely assumed, for reasons which need not be stated here, that the actual number of cells in the blood stream remains practically constant from hour to hour or even from day to day. A decrease in the proportion of blood cells in a unit of blood therefore signifies an addition to the fluid portion of the blood and hence an increase in the total volume. If the proportion of blood cells found at the beginning of any test or experiment be taken as the standard for comparison, the subsequent increase or decrease in the blood volume can be ascertained by dividing the percentage of blood cells in a given determination into the percentage found at the outset.

An increase in the proportion of plasma to cells signifies that the blood has become diluted and that the total volume of blood is therefore increased. Conversely, if the plasma is diminished and the cells per unit of blood increased, the blood is concentrated and its volume is diminished. The method of computation of the relative blood volume is illustrated in the following example:

The first examination shows: Blood cell volume = 50 per cent
 Plasma volume = 50 per cent

The second examination shows: Blood cell volume == 45 per cent
 Plasma volume == 55 per cent

Then the relative blood volume at the time of the second examination, is to the

*By using hirudin as an anticoagulant the blood cells settle to the bottom with extreme ease, and centrifugalization for a longer period than ten minutes at the above rate of speed, does not alter the final measurements and is therefore not necessary.

blood volume at the time of the first examination, as $45 : 50 = 100 : X$, or $\frac{50 \times 100}{45} = X = 111.1$. In other words the relative blood volume at the time of the second examination is 111.1 per cent of that of the previous one. If, on the other hand, the first examination shows the cell and plasma relations as above, and in the second examination the cells amount to 55 per cent of the total blood, the relative blood volume at the time of the second examination, is to that found at the first examination, as $55 : 50 = 100 : X$; or $\frac{50 \times 100}{55} = X$, i.e., $X = 90.9$ per cent.

In the first instance we find an increase, and in the second a diminution of the relative blood volume. Work is in progress which shows the necessity of controlling the quantitative findings of many ingredients of the blood by parallel estimations of variations in the relative blood volume.

The following protocols are supplied to illustrate the results obtained by this method:

TABLE 1.

Case L. K., I-4. Nov. 16, 1914. Coffee and glucose
50 g., 9:20 A. M.

Diagnosis: Hypopituitarism with diabetes.

Time	Blood cells Per cent	Plasma Per cent	Relative blood volume Per cent
9:15 A. M.	55.7	44.3	100.0
10:30	55.6	44.4	100.2
11:30	54.2	45.8	102.7
12:30 P. M.	55.5	44.5	100.4
1:30	55.5	44.5	100.4
2:30	55.7	44.3	100.0
3:30	55.7	44.3	100.0

TABLE 2.

Case L. F., K-16. Jan. 18, 1915. Coffee and glucose
50 g., 9:00 A. M.

Diagnosis: Diabetes mellitus.

9:00 A. M.	41.6	58.4	100.0
10:00	44.6	55.4	93.2
11:00	44.2	55.8	94.1
12:00	43.7	56.3	95.2
1:00 P. M.	44.3	55.7	94.0
2:00	43.5	56.5	95.6

TABLE 3.

Case A. M., K-1. Dec. 24, 1914. Coffee and glucose
50 g., 10:30 A. M.

Diagnosis: Diabetes mellitus.

9:30 A. M.	50.0	50.0	100.0
10:30	44.2	55.8	113.1
11:30	44.5	55.5	112.4
12:30 P. M.	44.0	55.0	113.6
1:30	42.6	57.4	117.4
2:30	42.9	57.1	116.5
3:30	48.0	52.0	104.2

TABLE 4.*

Cat 9.

Weight 3,750 gms. Complete pancreatectomy.

Time Hours	Blood**		Relative blood volume Per cent
	Plasma Per cent	Cells Per cent	
Before operation	64.3	35.7	100.0
After "	65.2	34.8	102.6
16	72.1	27.9	127.8
21	69.2	30.8	115.8
26	73.5	26.5	134.7
42	73.4	26.6	134.2
52	76.3	23.7	150.6
70	74.7	25.3	141.1
96	72.1	27.9	127.9
100	75.6	24.4	146.3
119	73.1	26.9	132.5
124	74.4	25.6	134.2
141	73.6	26.4	135.2

*Epstein, Albert A., and Baehr, George: Jour. Biol. Chem., 1916, xxiv, No. 1.

**Blood taken from ear vein.

TABLE 5.*

Cat 20.

Fasted 9 days before operation. Initial weight 3,300 gms. Final weight 2,880 gms.

Pancreatectomy and double nephrectomy.

Before operation	62.3	37.7	100.0
After operation	58.0	42.0	90.0
$\frac{1}{2}$	56.7	43.3	87.8
7	65.2	34.8	108.3
18	70.0	30.0	126.0
23	74.2	25.8	146.1
27	74.5	25.5	146.0
30	76.0	24.0	157.0
41	76.0	24.0	157.0
46	77.2	22.8	165.1
48	77.1	22.9	165.0

*l. c.

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EDITORIALS

Recent Organic Arsenic Compounds

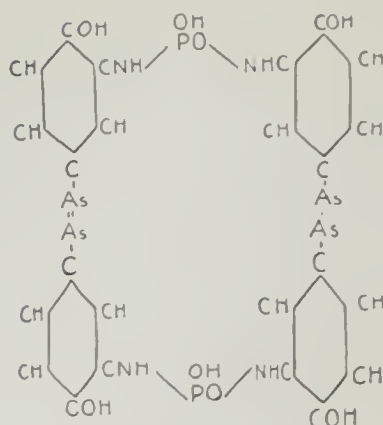
A STRIKING example of the great benefit which may result to practical therapeutics by the careful chemical, pharmacological and clinical investigation of new drugs is afforded by the recently introduced organic arsenic compounds. From the chemical standpoint certain peculiarly interesting and important features are manifested by these bodies. The first of these is the varying toxicity and often widely different symptoms which these bodies exhibit when compared with the results produced by corresponding quantities of arsenic combined in the inorganic compounds. There would seem to be involved here a peculiar combination of the action of the drug on the animal organism, and again of the reactions of the metabolic processes of the body on the drug. When parasitic organisms, such as spirochetes or trepanosomes, are present, as is usually the case in the conditions under which these drugs are administered, the conditions are still further complicated by the possible destruction of these organisms with the resulting liberation of toxic substances from this source. It is on this latter basis that a hypothetical explanation of the so-called Herxheimer (or Jarisch-Herxheimer) reaction which sometimes follows the administration of organic arsenic compounds in lues has been ex-

plained. In this condition there is swelling, edema, increased coloration or secretion, etc., about the local manifestations of the disease.

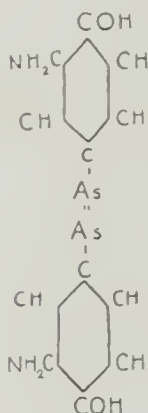
In most of its compounds the valence of arsenic is either three or five. And it seems probable that only the compounds of arsenic with oxygen¹ are capable of modifying vital functions. There are two oxides, the trioxide (As_2O_3), and the pentoxide (As_2O_5) of which the former possesses by far the greatest pharmacological action. This appears to depend on the trivalent arsenic atom. In the tissues of the body it is probable that each oxide is changed to some extent into the other, this being effected by reduction or oxidation of the pentoxide or the trioxide respectively. The ordinary salts of the acids of which these oxides are the anhydrides possess a corresponding action. In the tissues apparently the therapeutically effective arsenic exists in the form of arsenite ions, i.e., the trivalent form. This probably holds good as a general rule for the organic compounds also, for it was shown by Ehrlich that trypanosomes are not especially susceptible to the poisonous action of atoxyl in the test tube, but in the body atoxyl is very poisonous to these organisms. Ehrlich believed that in the tissues atoxyl was partially reduced and that these reduction products were the real trypanosome poisons, for he was able to show that such reduced bodies acted very powerfully on trypanosomes in the test tube. Others, however, are of the opinion that part of the atoxyl undergoes decomposition in the tissues and the ordinary inorganic arsenite ion is liberated and thus exerts its usual action. A further peculiar and therapeutically unfortunate circumstance in the administration of these arsenic compounds is that organisms such as spirochetes or trypanosomes tend to acquire a tolerance for the arsenic and, after a time, are no longer affected by therapeutic doses of the drugs. This appears as a rule to be more generally true in trypanosomiasis than in lues, for while the drug (atoxyl) may alleviate the symptoms of the disease, the cure of a patient with sleeping sickness is very rare.

The profound influence on humanity which a drug capable of curing such a disease as lues or trypanosomiasis may have has caused the greatest interest to be centered in the production and examination of organic arsenic compounds. And in addition, these drugs often appear capable of curing or alleviating diseased conditions other than those which were in the beginning the main objects of organic arsenic medication. Frambesia,² recurrent fever, Vincent's angina and spirillosis of the lower animals may be greatly benefited or cured by salvarsan, etc. And numerous favorable reports are in the literature regarding the use of these compounds in still other conditions.

A new impetus toward the chemical production of organic arsenic compounds has been given by the war, which has cut off from many countries the usual supply of salvarsan, neosalvarsan, etc. Accordingly a number of compounds, some new, others claiming to be the same as salvarsan, have appeared on the market. Among these are products from France, England, Canada, the United States, etc. Galyl³ is claimed to be tetraoxy-diphospho-amino-diarsenobenzene and to possess the following formula:



The formula of salvarsan is as follows:



In galyl there are thus introduced two phosphoric groups. It contains 35.3 per cent of arsenic and 7.2 per cent of phosphorus, is a yellow powder of a tint different from those of salvarsan and neosalvarsan, and is insoluble in distilled water. It, however, dissolves rapidly in a weak solution of sodium carbonate, from which it can be precipitated readily by the addition of an acid. The tubes in which it is dispensed contain the small amount of sodium carbonate required for solution, and thus the handling and dissolving are no more complicated than in the case of neosalvarsan. Foerster believes this drug to be as good as salvarsan in the treatment of lues, but admits that his observations have not yet extended over a sufficient time to permit a final judgment on the substance. Some disagreeable after effects often followed the use of the drug, but these were not severe.

Kharsivan and neokharsivan⁴ (English) are claimed to be identical with salvarsan and neosalvarsan as is also novarsenobenzol "Billion" (made by Pulenc Frères, Paris). There seems to be a general feeling among those who have used these drugs that they are somewhat more toxic than the original substances they were expected to replace. They may, however, with proper care be safely used.

Diarsenol as manufactured by the Synthetic Drug Company of Toronto, Canada, is said to be the dichlorhydrate of dioxidyamido-arsenobenzol. Apparently it differs only slightly from salvarsan. A number of reports⁵ have been made concerning the action and use of this body.

"Arsenobenzol" (Salvarsan) has been manufactured by the Department of Dermatological Research of the Philadelphia Polyclinic.⁶ This body (Arsen-

phenol-Amine Hydrochloride) is practically identical with salvarsan, but is slightly less soluble than the German product and requires to be filtered. It is prepared otherwise in the same manner as salvarsan and must be neutralized by the addition of sodium hydroxide solution. Ormsby⁷ and Mitchell have recently contributed an interesting report of 184 injections of this drug in 75 patients. They conclude that arsenobenzol, together with mercury, offers as good a method of treatment of syphilis as any heretofore used.

A number of other organic arsenic compounds⁸ have been introduced recently, but from published reports it is usually impossible to obtain accurate information regarding such therapeutic value as they may (or may not) possess.

It is easily seen that the ideal has not yet been reached in the production of these compounds. For aside from the difficulty of solution or of neutralization, etc., which several of them possess, there is the much more dangerous feature of their oxidation over into exceedingly poisonous bodies. In the case of the salvarsan and neosalvarsan there may be produced the dangerous paramidophenolarsenic oxide. This oxidation occurs rapidly in the air. The ideal drug should be stable, readily soluble, neutral in reaction, cheap and of low toxicity for the host while acting strongly on the parasites. It is to be hoped that the notable success of the past will continue to encourage greater efforts along these very promising lines in the future.

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—D. E. J.

Sensory Pathways in the Spinal Cord

INVESTIGATION of the exact function of the various tracts and centers that go to make up the complicated structure of the cerebrospinal axis is probably the most difficult problem in the whole domain of experimental physiology. The brilliant achievements of the morphological neurologists in tracing the origin and distribution of many of the tracts has not as yet been followed, to any material degree, by experimental evidence which would indicate the nature of the nerve impulses transmitted through them. Provided the function of the centers with which a certain tract connects is known, we can, it is true, draw inferences regarding the general nature of the impulses which must pass along it, but such inferences cannot be expected to carry us far when we consider, on the one hand, the paucity of our knowledge concerning the functions of the centers themselves, and, on the other, the diverse routes which may

be followed by the different groups of fibers that compose a given tract. Even if we could unravel to its last neurone the enormously complicated tangle of reflex arcs which compose the brain and spinal cord, we should know but little concerning the pathways along which different impulses travel. Thus, a pinprick on the sole of the foot will cause flexion of the homolateral hind limb (flexion reflex), whereas suddenly applied mechanical pressure at the same place will cause exactly the opposite effect, that is, an extension (extensor thrust). Both are reflex responses due to impulses traveling along practically the same reflex arcs, and yet the nature of the stimuli transmitted to the musculature of the leg is diametrically opposite in the two cases. Why the difference? It cannot be explained on any anatomical basis, and must therefore depend on conditions acting in the spinal cord in such a way as to direct and modify the entering sensory impulse so that it may either stimulate or inhibit the nerve cells of the motor neurones which, through the efferent roots, then transmit the impulses to the muscles concerned. In other words, to know the course of every nerve fiber and the position of every nerve cell in the cerebro-spinal axis would help us no more in determining through which reflex arcs any given afferent impulse will travel than would an exact plan of a telephone exchange indicate the connections that are most frequently made between its incoming and its outgoing wires.

In order to ascertain the nature of the impulses which a given tract transmits, or a center originates, we must study either the motor and sensory paralysis which supervenes upon its destruction or the movements and sensory impression produced by its artificial stimulation. The stimulation method is practically limited in its applicability to the surface of the brain, where, however, it has been used with great success in the localization of the so-called motor and sensory centers. To determine the function of the more deeply situated centers and tracts, the "method of destruction" is alone available, but even this has only a limited applicability, for although it can be used quite successfully in dumb animals to determine the course of motor impulses, it cannot obviously tell us much regarding the sensory pathways.

Practically all the knowledge we possess regarding the course of sensory impressions has so far been gained by accurate clinical observation of patients in whom a subsequent pathologic study has been made of the exact position of the lesions. But this method, besides being time-consuming, cannot carry us far, and when we come to investigate the problem on laboratory animals, we are limited to sensory impulses which cause some visible or objective response; something we can see and measure. Pavlov during the last years of his life employed for such a purpose the secretory activity of the salivary glands, and recently Ranson and von Hess have published the results of some excellent experiments in which reflex changes in arterial blood pressure were observed before and after destroying certain of the afferent pathways in the spinal cord. In these experiments, also, evidence was collected regarding the influence of the lesions on the conduction of painful or noci-ceptive impressions, this being the only sensation which, by the behavior of the animal, we can know to be present or absent.

Various lesions were produced on the spinal cord of anesthetized cats at

the level of the first lumbar segment, and after recovery from the immediate effects of the operation "each cat was subjected to two kinds of tests to determine the effect of the lesion, first, on the conduction of pain and, second, on the character of the vasomotor reflexes." The lesions consisted of dorsal and lateral hemisections, section of the posterior funiculus and destruction of the apex of the posterior horn. After recovery from the immediate effects of the operation, there was no evidence of motor paralysis (except in one case). The sense of pain as indicated by a sharp cry and generalized struggling movements was tested, usually by means of faradic stimulation through needle electrodes, in the area of distribution of the sciatic nerve. After inserting the necessary cannulae and while under light ether anesthesia, the vascular reflexes were elicited by faradic stimulation of the central end of the cut sciatic nerve, a pressor effect being obtained by strong and a depressor effect by weak stimulation. The influence of the spinal lesions on the reflex was gauged by comparing the magnitude of the change in blood pressure produced when the sciatic nerve was stimulated with that produced by stimulation of the central ends of the nerves of the anterior extremity. After these observations had been recorded, the animal was killed, and in order to determine the extent of the lesion a stretch of cord containing the lesion was removed, prepared by the pyridine-silver method of Ranson and cut into serial sections. By this method the presence of nonmedullated, as well as medullated nerve fibers, is revealed. In considering their results, the authors are careful to point out that all of the alterations in the vasomotor reflexes which were observed to supervene upon the spinal lesions which they produced cannot as yet be readily explained. The importance of their work lies, indeed, not so much in the actual conclusions which it is permissible to draw from the results, as in the great care and thoroughness with which the data have been collected.

Concerning the vascular reflexes, it was found that after lateral hemisection of the lumbar cord there occurred a great reduction of the depressor reaction elicitable by stimulation (weak faradic) of the opposite sciatic, whereas this reflex was practically normal when the sciatic on the same side as the cord lesion was stimulated. In the case of the pressor response, on the other hand, reduction was evident when either sciatic was stimulated, though more markedly so on the side of the lesion. These results suggest that depressor and pressor impulses travel in the spinal cord by different paths, of which the depressor are crossed and the pressor bilateral. Furthermore, the pressor paths are in the posterior portion and the depressor in the anterior portion of the cord, for in other animals in which posterior hemisections had been made it was found that the pressor reflex, but not the depressor, disappeared. The pathways available for the transmission of the pressor impulses in the posterior portion of the cord are the posterior columns (postero-median and postero-lateral) and the structures composing the tip of the posterior horn (Lissauer's tract, Substantia gelatinosa Rolandi, etc.). It was definitely established that it is by the apex of the posterior horn that the pressor impulses travel, for bilateral destruction of the posterior columns had no effect on the pressor reflex, which however could not be obtained after the apices of the posterior horns had been destroyed.

In connection with the last mentioned observations a most curious and in-

teresting result was obtained, namely, that the depressor reflex became very marked when the sciatic nerve was stimulated with such stimuli as would have caused a pressor response in a normal animal. This reversal of the normal reaction was also obtained, after destruction of the posterior horn, when the central ends of the brachial nerves were stimulated. To account for this result it is suggested by the authors that vasomotor centers, wherever these may be situated, are constantly being acted upon by afferent impulses which travel to them by different pathways, those coming up the lateral columns exercising an inhibitory or depressor influence, and those arriving by the posterior horns an augmentory or pressor influence. In normal animals, vasomotor reactions represent a balance between these two opposing influences. The facility of passage (threshold) of the depressor path is greater than that of the pressor, hence weak stimuli in normal animals cause depressor responses. Stronger stimuli, on the other hand, cause impulses to be set up in both pathways, but those with a pressor effect overcome the depressor impulses, so that the usual effect is a rise in blood pressure. The doorway guarding the depressor pathway is small and easily forced open, whereas the pressor doorway is large and unwieldy, but once forced open it offers less resistance to the crowd of impulses seeking passage along the pathway it guards.

After bilateral destruction of the posterior horns, "the pressor path is so impaired that with strong stimulation the inhibitory impulses along the depressor path predominate and produce marked depression." To explain why depressor responses should also be obtained in these animals on strong stimulation of the brachial nerves, it is suggested "that the normal high resistance of the pressor path has been increased above the lesion by cutting off the impulses normally ascending in this path from the lower half of the body."

The depressor pathway seems to be that along which painful impulses and those that cause reflex changes in respiration are conducted; at least, it could be definitely shown that the pressor pathway is not concerned in the transmission of such impulses, for none of the above mentioned lesions had any noticeable effect on the conduction of pain. This conclusion is in conformity with the usually accepted one that pain is transmissible up the cord in the anterior part of the lateral funiculus.

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—J. J. R. M.

Acidosis in Nephritis

HALDANE and Priestly showed that the regulation of respiration is effected by the carbon dioxide tension of the arterial blood which is the same as that of the alveolar air of the lungs. Later work showed that the hydrogen-ion concentration due to the dissolved carbon dioxide is the actual exciting agent. An increase of 0.22 per cent of the carbon dioxide content of the arterial blood results in doubling pulmonary ventilation. But carbon dioxide is

not the only substance which acts in this way, as has been proved by various investigators. Minute additions of hydrochloric acid will produce similar results. Lactic acid and undoubtedly other acids will act in the same way.¹

While it is the lungs which are the chief organs for the regulation of the carbon dioxide content of the blood, it is the excretory organs, which govern the excretion of other acid products of metabolism. Bayliss remarks that the sensibility of the kidney to acid in the blood must be such as to keep the concentration of hydrogen-ion in the blood, other than that due to carbon dioxide, at a constant level.

Dyspnea may therefore be of one or another of two types,—that due primarily to increased carbon dioxide tension in the lungs and blood, and that due primarily to increased content of other acids in the blood. It is this latter type of dyspnea which led Peabody to study the acidosis of nephritis.²

Recent studies upon the blood in uremia have tended to show that this condition is frequently, especially in its advanced stages, associated with an acidosis which is sufficiently marked to alter the normal composition of the blood and to reduce the carbon dioxide tension. In chronic nephritis without uremia, investigations of the blood and alveolar air have shown little evidence of acidosis. That there is however a certain degree, often small, of acidosis in ordinary grades of nephritis, has been shown by Sellards,³ who used what is known as the alkali tolerance test. (This test, credited to both Sellards and Palmer,⁴ depends upon the fact that in a normal individual 5-10 grains of sodium bicarbonate taken by mouth causes a change in the reaction of the urine from acid to alkaline in about 2 hours. The test is applied by giving a patient 5 grains of sodium bicarbonate every 2 hours until the urine becomes alkaline to litmus. The amount of the bicarbonate necessary to produce the urinary change indicates the degree of the acidosis.) When the acidosis rises above a certain grade it is indicated by dyspnea, which is the physiologic expression of stimulation of the respiratory center.

Peabody notes that he and other workers have discussed the association of acidosis and dyspnea in cardiorenal disease, but that they have not been able to make clear the relation between the two symptoms. He remarks that the usual clinical laboratory examinations of the urine are not satisfactory because they give information only of the acid substances which are being excreted, and give no information as to how much is retained. This information may be had by making blood acidity studies supplemented by estimations of the carbon dioxide tension in the lungs. However that may be, all the *necessary* data may be had by the use of alkali tolerance test, which naturally will tend to show how much excess of acid there is within the body.

Peabody compares three groups of cases. The first consists of patients who gave evidence of a definite but not severe nephritis, as shown by urinary findings with, usually, hypertension and cardiac hypertrophy. In this group the alkali tolerance, phenolsulphonphthalein output, and alveolar carbon dioxide tension fell within normal limits. In other words, in this series of mild chronic nephritis there was no evidence of acidosis. The second group comprised cases in which the phenolsulphonphthalein output and nitrogen retention determinations were abnormal. In these the alveolar carbon dioxide determination were

normal but the alkali tolerance test required more than the usual alkali. In this series it is noted that the alkali tolerance and the output of phenolsulphonphthalein run parallel. These findings signify an early stage of acidosis without increase of alveolar carbon dioxide tension. The third group consisted of advanced cases of chronic nephritis. Six of the nine died within a few days or weeks of the observations. All the tests indicated low functional activity (phenolsulphonphthalein) of the kidneys. Alkali tolerance was increased and the alveolar carbon dioxide tension was decreased. In these cases, says Peabody, there is not only evidence of loss of base from the tissues, but also of increase of acids in the blood. These facts, he says, point to the acidosis of renal disease, being due to retention, or to inefficient secretion.

This work is exceedingly interesting and calls attention to important diagnostic and therapeutic procedures which have been dwelt upon very logically and impressively by Martin Fischer.⁵ But Peabody's results do not go back far enough. That the acidosis is one due to retention is interesting, but not so interesting or important as the cause of the retention which Fischer has studied. In plain uncomplicated nephritis, there is edema of the kidney due to a local renal acidosis. Excretion may be cut down or not, and be a serious factor or not, according to the degree of renal involvement. When the acidosis of nephritis is more general then the edema is also more general and other tissues than the kidney become affected,—acids are held by all the organs according to the character of their arterial blood supply. When the brain becomes edematous during a nephritis, uremia results. In other words, isn't the cause in all cases much the same, and do not the symptoms vary with the organs involved?

That acidosis is but one factor in uremia is very probable, but also it is probably the most important single one, as alkali therapy indicates. It is noted that by alkali therapy Peabody means the administration of moderate intravenous doses of 5 per cent sodium bicarbonate solution. Such a solution does practically but one thing; i.e., it combines acid. When one considers what is happening in the acidosis of uremia, one must realize that the problem is not entirely one of acid content of the tissue though that is the primary thing, but it is water holding by the tissue. To reduce that needs more salt content, even in uncomplicated cases. In nephritis it is possible that the acids are not however the usual ones such as lactic acid and the rest, but they may be amino acids and to counteract the effects of these the alkaline salt solutions are not as efficacious as they are in the more usual types, in which they produce "great temporary improvement." In the group "in which acidosis only produces a slight fall in alveolar carbon dioxide tension, and in which there are no symptoms directly due to the acidosis, the results obtained by giving alkali are much more questionable," probably because of the type of acidosis. There is every probability that in the amino-acid acidosis alkali treatment is not as essential as sugar treatment. In other words, it is possible that by using a salt-alkali treatment on the one hand, or a dextrose or lactose containing salt-alkali treatment on the other, that the two types of acidosis might be separated.

It seems worth the saying that in very many chronic nephritides the vascular lesions are the important ones. In many cases of "uremia" the cerebral vessels are essentially diseased. When this is true, it becomes an important therapeutic

question to determine how much salt or alkali or other substance can be introduced to at once decrease the swelling associated with increased acid content, and to neutralize the acid. Neutralizing the acid in the blood alone will give only a transient effect. If the conditions are such that without a decreased carbon dioxide tension in the alveoli there is nevertheless a cerebral or a renal acidosis (swelling), it means that the available oxygen of the blood does not reach the organs, and, under such conditions, intravenous medication will always be only an experiment.

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—P. G. W.

The Bacillus of Plotz and Typhus Exanthematicus

THE repeated outbreaks of typhus fever which have marked the epidemic history of the present war promise to furnish the opportunity for final settlement of the problem of the etiology of this disease. Systematic scientific investigation of typhus dates from 1909 when Nicolle succeeded in inoculating anthropoid apes. Very soon after this, Anderson and Goldberger, Ricketts and Wilder, and Gavino and Girard successfully inoculated lower monkeys, and material for systematic experimentation was thereby made available. The definite temperature reactions in guinea-pigs inoculated intraperitoneally with typhus blood in the early stages of the disease were then reported by Nicolle Corneil and Conor, and this has been extensively confirmed by Anderson and many others, Anderson carrying a typhus virus in this way through many generations. The filtrability of the virus, though at first claimed by many observers and still held by some, was gradually shown to be unlikely by Anderson and Goldberger. Ricketts and Wilder, and, although occasional monkeys inoculated with filtered virus have proved refractory to subsequent inoculation, the general opinion to-day is that the disease is not caused by a virus too small to pass the filters. This of course has given new impetus to the search for bacterial causative agents.

Many microorganisms have been described. Marcus Rabinovitch¹ described a Gram positive diplobacillus which he cultivated from cases in an epidemic in Kieff and with antigens prepared from this organism he obtained complement-fixation and agglutination. Fürth studied an epidemic in China and obtained short, plump rods which grew aerobically in short chains, which were first looked upon as diplococci. P. Th. Müller saw a diplobacillus upon which he did not lay much stress etiologically, and von Prowezek described inclusions in leucocytes which he regarded as protozoa. It is hardly worth while at the present time to describe in detail the many different findings that have been reported, since in few of them is there sufficient evidence to enable us to come to conclusions.

In 1914 Plotz² described a short Gram positive bacillus which he obtained

by anaerobic cultivation, with much regularity, from cases of Brill's disease at the Mt. Sinai Hospital, New York, and which has been made the subject of considerable study by Plotz, Olitsky and Bachr.³ They have obtained the bacillus again and again, have succeeded in obtaining positive agglutination and complement-fixation in the blood of endemic typhus cases after the crisis, and have obtained a similar bacillus from a number of European typhus cases which have come into quarantine.

The method of cultivation by which this bacillus is obtained is relatively simple, consisting of taking blood directly from a vein into high tubes containing glucose agar and unheated and unfiltered ascitic fluid of a specific gravity not less than 10, 15. The American Red Cross Commission which went to Serbia during the last typhus epidemic—and of which the writer was a member—attempted to work along the lines laid down by Plotz but found it extremely difficult to do systematic work and obtain reliable materials under the conditions then existing. The undersigned obtained an organism very similar to the Plotz bacillus by Plotz's method in two cases. In the first of these the organism could not be carried further than the second generation, and in the second it did not reach America alive. Hopkins obtained a similar organism later toward the end of the epidemic, which is still under cultivation. However these organisms were found so rarely in spite of a considerable number of blood cultures that were taken, and the necessity of working with unsterilized ascitic fluid under relatively primitive conditions compelled the judgment that these isolated findings, though pointing somewhat in favor of Plotz's organism, could not be taken as warranting conclusions. However, of the many blood cultures taken, the smaller number were taken from fresh cases, most of the ascitic fluid used did not come up to the specifications of Plotz, and there is no doubt about the fact that the organism described by Plotz is not one that is easily cultivated unless rich ascitic fluid is available.

The fact that the Plotz organism has been cultivated from guinea-pigs and monkeys experimentally inoculated by the workers at Mt. Sinai, as well as by Anderson, is further evidence in favor of this bacillus.

Petruschky⁴ has recently cultivated a similar but aerobic bacillus from sputum in typhus cases and Arnheim⁵ has cultivated an organism which in appearance and staining properties is not unlike the Plotz bacillus, but also grows aerobically. Arnheim obtained his organism from six cases, on ascitic agar plates, on which on the first cultures there appeared a growth hardly visible to the naked eye, which in transplants continued to grow very delicately. He states that his organism is not unlike that of Petruschky and that he obtained it out of the blood, the sputum and the urine of typhus cases.

Judgment on all these many different findings is complicated by the fact that the undersigned, as well as others, has found that typhus cases in the later stages of the disease are often secondarily infected and streptococci and other organisms can occasionally be cultivated from the blood in the later stages and from the spleen at autopsy. Moreover the fact that the organisms most persistently described are in appearance and staining properties so similar to the so-called *diphtheroids*, which are so often obtained, with doubtful etiological significance, from lymphoid tissues, blood and ascitic fluid intro-

duces an element of doubt which should lead to the greatest conservatism. This applies, of course, more strongly to the organism isolated by Plotz, in which it is apparently indispensable to use unheated and unfiltered ascitic fluid. However, in the work of Plotz, Olitski and Baehr, this element seems to have been very carefully controlled by many blood cultures taken in nontyphus cases with the same ascitic fluid used in the blood cultures taken from patients.

Of all the organisms so far under consideration, that of Plotz seems to be the most important, but final judgment must await results obtained in the work now being carried on by Plotz and others thoroughly familiar with the method, in Europe and in other places.

The fact that the organism within one generation seems to lose entirely, or almost so, its virulence and that so far vaccination of animals has not protected them against inoculations with the virus is a point which justifies reasonable doubt. On the other hand, failure to vaccinate, or even to actively immunize, is not conclusive proof against the etiological significance of an organism. It seems established with fair definiteness that a bacillus like that of Plotz can be cultivated from many cases of typhus exanthematicus and there seems to be a strong possibility that the organism causes the disease. The work that has been done on it has been done with great care and a desire to get at the truth, and the work at present being continued in this spirit, with available typhus material in Europe, probably by a number of independent workers familiar with the disease and under conditions of well equipped laboratories, should bring conclusions which before long will permanently settle the question of the etiological significance of the Plotz bacillus.

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—H. Z.

Tumor Problems

THE fact that very many laboratory workers in all parts of the world are studying tumors, and that the experimental results are published in very many journals, many of which are inaccessible to men who are not spending most of their time in laboratories where the literature is accessible, makes a general discussion of the problem of cancer research of considerable value. Such a discussion is furnished by Ewing.¹

Ewing comments at some length upon the problems presented during researches upon the transmissibility of animal tumors. A prominent problem, he says, is whether all of the tissues used in such experiments are true neoplasms, and in connection with this he quotes Virchow's remark, which many investigators and students forget, to the effect that no one, even under torture, can state exactly what a tumor is. The older notion, that a true neoplasm is only viable in its host, we now know is not true, and this fact makes it increasingly difficult to separate the infectious growths from the noninfectious. The morpho-

logic criteria (and these are practically the only ones we have as yet) of malignancy are the following:

First, and most important, the atypical qualities of tumor cells. In the absence of other characters this alone, when sufficiently developed, determines the fact of malignancy. The degree of variation from the normal type of cell from which the growth originates is what one means in this instance.²

Second,—the loss of polarity. In normal tissues there is a regular relationship of the cells to one another, or to basement membranes, which is lost as cells become atypical and take on malignant characters.

Third,—infiltration, by which is meant the power of invading and in part destroying the invaded tissue.

Fourth,—metastasis, which is usually pathognomonic of malignancy, but which may occur in almost any type of benign growth.

Fifth,—the capacity to excite the growth of connective tissue. This property denominated desmoplastic, is not constant.

Sixth,—hyperplasia, a symptom which is difficult to delineate because of the grades of hyperplasia which result from irritation and functional causes.

Judged by these criteria, says Ewing, the tumors of the lower animals are true neoplasms. Speaking of the Rous chicken sarcoma, he seems to have no doubt. The fact that this growth can be transmitted by using filtrates from porcelain filters, is interesting but not conclusive that the process is infectious. Experimental criteria for neoplasms include bacterial sterility and inability to be propagated by filtrates. Rous' tumors are sterile. Ewing believes that in the case of these tumors one is dealing with a chemical transmissible virus. This point of view in its application to tumors is an exceedingly interesting one for which undoubtedly much might be said. It will be sufficient, however, to merely mention the fact that there is in the work on "Vitamines" evidence that exceedingly small amounts of matter may be able to produce exceedingly large effects, and that it is entirely possible that in the case of tumors there is a substance (or substances) which even in infinitesimal amounts may incite cells to growth and continuous overgrowth. It is possible that it is the physical condition of such substances which accounts for their activity rather than the mere chemical structure.

Ewing calls attention to another point of view that deserves accentuation. This refers to the possibility of variation of standards for animal and human tumors, examples of which possibility he cites. He refers briefly to other aspects of the cancer question, such, for instance, as the effects of constituents of dietaries,—carbohydrates, lime; and to the effects of x-rays and radium. In speaking of the influence of age, he refers to the fact that whereas cancer has been considered a disease of old age, nevertheless young animals have been found to furnish a better soil for the growths in experimental work. He refers to the problem of associated lymphocyte change and the growth of tumors.

This review of cancer problems, Ewing says, inclines him to believe that each neoplasm should be considered as a specific disease, each type differing in its etiology, clinical course and therapeutic possibilities. He compares the neoplasia as a group with the infectious diseases as a group, and points to the fact that in the latter the general features may be alike yet the causes are different.

We might note especially the similarities in the group of granulomas. It may indeed be possible that though the carcinomas appear alike, they are etiologically dissimilar. Perhaps it is for this reason that excision is the only satisfactory method we have yet of dealing with tumors, and that other therapeutic measures have failed in universal useful application.

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—P. G. W.

Recent Developments in the Therapeutics of Lobar Pneumonia

IN the study of few of the common bacterial infections has there been more cooperative activity of recent years than in that of lobar pneumonia. The intensive study of pneumococcus immunity by Neufeld, a study covering many years and optimistically continued in spite of many discouragements, finally yielded important results in 1910. Neufeld and Haendel were able to show that an important element in the failure of previous workers to influence pneumococcus infections by serum therapy had depended upon the fact that the pneumococcus group included more than one organism, and that the different types, though morphologically and culturally entirely alike, were serologically strictly distinct. The antibodies produced in an animal by immunization with one strain had practically no activity upon cultures of another strain. In consequence, no serum therapy could be expected to exert any influence unless a serum was used which had been produced with a strain of organisms identical in type with that infecting the patient.

This line of investigation has been followed extensively at the Rockefeller Hospital, where in the laboratory of Cole, Dochez and Gillespie succeeded in making a preliminary classification of pneumococci into four types. Type I and Type II are apparently well-defined groups, the members of which react specifically with sera of their respective types. Type III is the pneumococcus mucosus and Type IV is a temporary "attic" into which heterogeneous types not reacting with sera I or II and not interrelated among themselves are conveniently stored for the present. Types I, II, and III have been found to be associated most frequently with disease, whereas Type IV, though also very common in pneumonia, is nevertheless less common than the preceding and apparently less serious prognostically. A serum powerful enough to influence infections with Type I has been successfully produced, and this serum, injected intravenously, while apparently not influencing the process in the lung, nevertheless will sterilize the blood and therefore exert a powerfully beneficial action in severe cases. Although the serum treatment of Type I pneumonia is still more or less in the experimental stage, it is being extensively used at the Rockefeller Hospital and in other hospitals which have access to the serum there produced. And since in the opinion of Cole and others who have employed it, the use of the serum represents a probably very important advance in therapy, it becomes very important for hospital laboratories and practitioners of medicine to be familiar with the method at present in use for the determination of the pneumococcus type.

The method at present in use and based on the work carried out at the Rockefeller Hospital is as follows:

The patient is required to wash out his mouth with some antiseptic, and then expectorate sputum, obtained by coughing, into a Petri dish. The sputum is washed by lifting it through a series of watch glasses containing sterile salt solution, is then ground in a mortar with a little salt solution, and the even emulsion so obtained is injected intraperitoneally into a white mouse. A fine needle is carefully introduced upward and inward from just above Poupart's ligament. Puncture of the liver may be avoided in this way. The pneumococci develop in the peritoneum, and after four to eight hours the mouse is killed and the peritoneal cavity carefully washed with salt solution by means of a capillary pipette. The washings are placed in a centrifuge tube and centrifugalized at low speed for several minutes to bring down leucocytes. The supernatant fluid is then centrifugalized at high speed and the pneumococci so obtained are resuspended in salt solution. This suspension is added to equal parts of the sera of Types I and II in dilutions of 1 to 2½ and 1 to 10, with, of course, proper controls and a bile test to determine bile solubility. Agglutinations can also be made later with broth cultures made from the mouse's heart's blood or peritoneal exudate.

The agglutinations can be confirmed by protection experiments in mice, but this is usually unnecessary in hospital work, since the agglutinations sharply determine whether the organisms belong to Type I or II, or, by elimination, to Type IV. If of Type I, serum treatment can be instituted. In the case of Type II, it is not improbable that such treatment may be possible in the near future. If the organism belongs to Type IV, a certain amount of prognostic information is gained.

—H. Z.

Diuresis

THE mechanism of diuresis is unsolved, probably because the mechanism of urinary secretion is unsolved. It may perhaps be possible that light upon this subject may be shed by studying diuresis and applying the discovered facts to urinary secretion.

It has been assumed that during the secretion of urine a protein-free aqueous solution of salts is formed in the glomeruli and that this solution in its passage through the renal tubules becomes concentrated by reabsorption of water, and by the addition of urea, uric acid and certain salts.¹ It has been assumed, on the one hand, that the fluid in the glomeruli is a pure filtrate, or, on the other, that it is formed as an expression of secondary activity of the glomerular epithelium. The one assumption necessitates the action of pressure; the other postulates a factor or factors of which nothing is known, unless we engraft upon it a physicochemical conception which takes account of absorption. Against the filtration theory stands the fact that there never is sufficient pressure difference to permit of rapid enough transmission of water from a colloidal sys-

tem like the blood, through a colloidal membrane such as the glomerular capillary walls.

The more recent conception of Fischer² seems for the present at least, to take account of all the conditions in a more satisfactory manner than the older theories. According to Fischer, urinary secretion depends essentially upon the presence of free water in the blood of the kidney; that only when the water is free can it pass the colloidal walls of the renal capillaries or tubules under the conditions existing in the kidney. If water in colloidal combination be added to the blood, as, for instance, in the form of blood serum, urinary secretion is not affected to any appreciable extent no matter how much serum is introduced. This is the basis of Hogan's treatment of shock.³ But even if there is free water in the blood, little of it will pass into the glomeruli unless the chemical conditions in the various colloids in the kidney are properly balanced. If there is too much acid in the blood, the water will be held more closely to that fluid; if the acid content of the blood falls; i. e., if the oxygen content is raised, the water will become free for excretion. In other words, it is only when the tissue of the kidney which borders on the blood has a relatively higher acid content than the blood itself that water can be taken from the blood and started in its excretory path. If the blood flow through the kidney is slow and the oxygen content is low, secretion also will be low; if it is rapid, and the blood is well aerated in the lungs, the oxygen content of the arterial blood in the kidney will increase, the acid content will be lower, and secretion will increase. The diuretic drugs, such as caffeine, act by increasing the arterial flow through the kidney. The diuretic salts act by counteracting the effects of acids in the tissues.⁴

If the colloidal theory of secretion is true, then all substances which produce diuresis must do either or both of two things; they must increase the arterial blood supply through the kidney, or they must act in a physicochemical way upon the renal colloids and cause them to give up water.

Cow⁵ has recently observed that physiologic amounts of saline solutions, injected into the blood stream, produce no increase in diuresis, while if the same amounts are given by mouth a diuresis occurs. This led him to suggest that the water taken by mouth accumulated some substance from the mucosa of the gastrointestinal tract, which had a diuretic effect, and that that substance, directly or indirectly, is largely concerned in the production of diuresis. He observed that extracts of duodenal mucous membrane, when used for perfusing the kidney, caused a diuresis, provided the perfusion fluid previously had been pressed through the vessels of the head and neck. Passage through a headless animal was ineffective. These observations suggested that the pituitary secretion played a part in the problem. The process, as Cow conceives it, would be something like this,—water enters the gastrointestinal tract and during absorption takes something from the mucosa of the duodenum. This in turn is carried to the pituitary which is stimulated to produce its substance which has an effect upon the urinary secretion. This is an interesting contribution which, as has been suggested⁶ recalls the mechanism of pancreatic secretion. But after all it merely states the possibility that extracts of duodenal mucous

membrane may have an effect in causing the pituitary to secrete. Emotions of one or another type or grade may do the same thing. It helps the problem of renal secretion not at all. It, however, seems to emphasize the fact that renal secretion is a chemical problem and that it does not depend upon the nerves. In partial support of this stands the work of Pearce and Carter⁷ who have shown that so far as the vagus is concerned, the oxygen consumption of the kidney is not changed by stimulation of that nerve. This work also shows that renal activity is primarily dependent upon the use of oxygen.

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—P. G. W.

Chloroform Necrosis of the Liver.

IT has been known for some time that chloroform necrosis may be followed by pathologic changes in the liver, and by a series of symptoms which simulate acute yellow atrophy. It is known from the writings of Wells,¹ Whipple,² Graham³ and others, that these changes depend upon the fact that chloroform reduces the oxidation processes of the body. In connection with the work upon this subject it was reported first by Whipple² that whereas adult experimental animals were susceptible to chloroform, and became the subjects of chloroform liver necrosis, young animals, i.e., ones within three weeks of age, were not susceptible. Graham⁴ has attempted to discover why there is this difference between adult and young animals. He has been able to corroborate Whipple's observation of the relative insusceptibility of pups, and feels that he has evidence that this insusceptibility is due to the glycogen content of the livers of the animals. This evidence depends upon the fact that starvation or phlorhization which depletes the glycogen, produces pups which show liver necrosis after chloroform; that increasing the glycogen content of the liver of adult animals renders them less susceptible to chloroform necrosis; and that in well-nourished pups the glycogen content of the liver may be as high as 9.07 per cent.

In another article Graham⁵ shows that chloroform necrosis depends upon the formation of hydrochloric acid from chloroform, but he is not able to account for the inhibitory effects of glycogen. And yet the experiments which he gives are convincing and lead to the very important therapeutic suggestion that the feeding of carbohydrates to adults lessens their susceptibility to liver necrosis by chloroform.

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—P. G. W.

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ORIGINAL ARTICLES

POISONOUS PROTEINS*

BY VICTOR C. VAUGHAN, M. D., ANN ARBOR, MICH.

INTRODUCTION.

ORIGIN of Proteins.—In nature all proteins are the products of life and each kind of living molecule elaborates and contains its own specific protein. Some forms of life are capable of constructing their proteins out of inorganic matter, while others can utilize only that which has been built up by other cells into protein material. Plants take the ammonia, nitrates and nitrites of the air, soil and water and by synthetical processes convert these into the proteins found in their tissues. In this process there are two stages. In the first the inorganic nitrogen is synthesized into amino acids and in the second these are combined to form proteins. The higher animals cannot synthesize inorganic nitrogen into amino acids. This is done for them by plants and to some extent by bacteria in conjunction with plants. By the symbiotic action of certain bacteria and plants even the free nitrogen of the air is drawn upon in the construction of vegetable proteins. So far as protein metabolism is concerned the vegetable world is the synthetical or constructive laboratory while the animal is the analytical or destructive machine. The plant takes the smallest parts and builds them up into highly complex bodies, while the animal takes the complex and splits them into pieces to be reconstructed in its own body. In a general way the above statement is true, but there are synthetical processes going on normally in the animal body and it is demonstrable that simple proteins may be built into more complex molecules in the animal body. Moreover, it is certainly true that in man with perfect digestion practically all the nitrogen of the food is absorbed in the form of amino acids. The animal as well as the plant is a synthetical laboratory, but the new material used by the former is the finished product of the latter, which is unravelled and then woven into a new pattern which is different in each species of animal.

*The Herter Lectures for 1916 given in the University and Bellevue Medical School, New York.

There are as many kinds of proteins as there are kinds of living matter. Chemically proteins are polymers of amino acids. The amino acids demonstrated in proteins are only about eighteen in number, but with these put together in an almost infinite variety of ways, we get an unlimited variety of products, just as with only twenty-six letters in the alphabet there is no end to the making of words. The simplest proteins consist wholly of amino acids. These combine with inorganic salts, lime, phosphorus, iron, etc., and with carbohydrates to form the compound proteins.

All living things not only contain protein, but this is their essential constituent. The living protein molecule is in a labile or active state, capable of trading in energy, absorbing and eliminating; never in a condition of equilibrium. Dead protein is in a state of rest; it is a stabile molecule and remains in equilibrium.

BACTERIAL PROTEINS.

Material.—Fifteen years ago, after various attempts to secure bacterial cellular protein in large amount, I succeeded with the tanks for massive cultures which have been described elsewhere. The growths thus obtained are freed from extraneous matter by washing with dilute alcohol and then by thorough successive extractions with absolute alcohol and ether. The cellular substance is ground first in porcelain and then in agate mortars, and passed through fine meshed sieves. Whatever the bacterium employed the product is a fine white powder. The dilute alcohol removes the extraneous matter mechanically held by the growths and the long continued extractions with alcohol and ether remove coloring matters, fats, waxes, and other less known bodies. I have never made a close study of these extractives. These bacterial powders when examined microscopically show the individual cells plainly, especially when properly stained. Even the chromogenic bacteria come through as white powders, all the color being removed by the alcohol and ether. The freedom of this cellular material from extraneous matter is best appreciated from the fact that when one gram of it is incinerated there is no trace of chloride in the ash. With chloride of sodium in the culture medium and considering the ease with which traces of chloride are detected, this indicates a surprising degree of purity in the material. The significance of the absence of chloride will be discussed later. The cellular protein of many pathogenic and nonpathogenic bacteria has been obtained by growth in the tanks. I may say that I have never dared to grow anthrax bacilli in these massive cultures and have contented myself so far as this organism is concerned with the less abundant growths in Roux flasks. For obtaining abundant growths of the tubercle bacillus the tanks are less suitable than glycerine beef-tea cultures.

Chemistry.—It has been generally assumed that bacteria are unicellular plants. This assumption rests, so far as I can learn, upon early statements, such as that of Pollender, that anthrax bacilli are not affected by strong alkali and this has been interpreted as meaning that they consist largely of cellulose. It is true that certain investigators have claimed to demonstrate even large amounts of cellulose in bacteria. Hammerschlag on wholly inadequate evidence estimated the per cent of cellulose in the tubercle bacillus as high as 28.1. DeSchweinitz and Dorset reduce this amount to 6.95 per cent, but hardly accept this figure

themselves since they conclude that cellulose is probably present in small amount in the tubercle bacillus, and not present in the bacillus of glanders. These and other investigators, who have reported the presence of cellulose in bacterial cellular substances, have not properly distinguished between cellulose and other carbohydrates. Vincenzi employing proper tests failed to find cellulose in the cellular substance of bacillus subtilis, but did find a nitrogenous carbohydrate. In our work, Wheeler made special search for cellulose in sarcina lutea. Twenty grams of cell substance was autoclaved with 25 parts (500 c.c.) of ten per cent potassium hydroxide at 120°, first for thirty minutes and then for an hour. There remained a considerable residue which gave none of the protein reactions, did not reduce Fehling's solution even after prolonged boiling with dilute hydrochloric acid, but did respond to the carbohydrate test with alpha-naphthol. Cellulose could not be detected by any known test. Schweitzer's reagent failed to dissolve it and it gave no color with iodine even after treatment with sulphuric acid. A portion was dried and heated with soda lime when it evolved a gas which turned red litmus paper blue, thus indicating the presence of nitrogen which had been reduced to ammonia. The odor of burning feathers also indicated the presence of nitrogen. From these results we conclude that there is no evidence of the presence of cellulose in bacterial cellular substance. Leach made search for cellulose in the cells of the colon bacillus with like negative results. Like Vincenzi we did find a nitrogenous carbohydrate. This is chitin or some chitin-like substance. The presence of chitin in bacterial cell substance has been reported by Ivanoff, Emmerling, Helbin, Bulloch, and others.

My students and I have found two carbohydrates in bacterial cellular substance. One, referred to above, is combined with nitrogen, is not soluble in strong alkali, and does not reduce Fehling's solution even after prolonged boiling with dilute mineral acids. The second carbohydrate is combined with phosphorus, is soluble in alkali, and does reduce Fehling's solution after being boiled with dilute mineral acid. In the unbroken molecule this carbohydrate undoubtedly is contained within the nuclein group. If the presence of cellulose be essential to plant tissue, bacteria certainly are not forms of plant life.

There is no controversy concerning the presence of nuclein in bacterial cellular substance as the xanthine bases have been demonstrated among the disruption products both in my own laboratory and elsewhere. The literature on this subject is too extensive to permit me to go into it exhaustively and I will content myself with a few references. Klebs obtained from the tubercle bacillus a nuclein containing 8-9 per cent of phosphorus. From the same organism Ruppel separated a nuclein containing 9.42 per cent of phosphorus which he designated tuberculinic acid. Levin obtained from the tubercle bacillus proteins, nuclein, and crystals which he considered a mixture of thymil and uracil, also cystosin. Lustig and Galeotti obtained a nucleoprotein from the pest bacillus. In our work the presence of nuclein bodies was plainly in evidence. Leach obtained from the colon cell substance a body containing 7.33 per cent of phosphorus and both Leach and Wheeler secured evidence of the presence of xanthine bases.

Bacterial cellular substance responds to all the protein reactions. Proteins are detached from the substance by both alkalis and acids, but the properties of the bodies thus obtained indicate that they are split products obtained by the

cleavage of more complex molecules, and do not exist free in the cellular substance. Dilute mineral acid splits off the nitrogenous carbohydrate and when this extraction is carried on at high temperature much of the second carbohydrate is converted into a reducing substance. The acid extracts when dropped into a large volume of alcohol give a precipitate which after purification by resolution in water and reprecipitation with alcohol yields more than seven per cent of phosphorus. The line of cleavage through the large molecules in the cellular substance followed by acid action seems to be definite and the same products are obtained with one per cent and 0.1 per cent sulphuric acid. More concentrated acids after prolonged heating break deeper into the molecular structure and cleave the biuret bodies with the liberation of amino acids. Wheeler and Leach have made special studies of the action of mineral acids on bacterial cellular substance. Ten per cent solutions of potassium hydroxide at 120° extract from bacterial cell substance everything except the chitin-like body which consists of a carbohydrate combined with nitrogen.

We have demonstrated the presence in bacterial cellular substance of both mono and diamino acids and have shown that the percentage of these varies with the microorganism. We have found the percentage of nitrogen to vary from 5.964 in *subtilis* to 11.765 in *violaceus*.

I began this work with the expectation of finding the bacterial cell substance composed of relatively simple bodies. I have been compelled to come to the opposite conclusion. The cellular substance of bacteria contains highly complex molecules. We have demonstrated the presence of the following groups among the split products: (a) a chitin-like body consisting of a carbohydrate combined with nitrogen. It seems reasonable to infer that this exists in the cellular substance as a glyco-protein. (b) A carbohydrate group combined with phosphorus from which it is not easily detached. This group reduces copper after prolonged boiling with dilute mineral acid. The amounts as determined by the reduction of Fehling's solution and calculated as xylose are large, but we are not sure that the reducing substance is all carbohydrate. Indeed, it might be better to speak of both of these groups as those responding to the Alpha-naphthol test rather than as carbohydrates and to distinguish between them as nonreducing and reducing bodies. However, it seems clear that the one now under consideration is a subgroup in the nucleinic acid constituent of the cell substance. (c) The presence of nucleinic acid is beyond doubt, as is shown by the high phosphorus content of some of the split products and by the demonstration of the xanthin bases. (d) That one or more protein groups exist in the cell substance. If all these groups exist in the same molecule the cell substance must contain a highly complex molecule which would be best designated as a glyco-nucleo-protein. The fact that these bodies are removed only by agents capable of causing molecular disruption inclines me to the belief that the molecules which make the cell substance are highly complex. It may be said that this is an assumption and without adequate proof. On the other hand, such a statement as that made by Doerr, that bacterial proteins are of simple molecular structure is wholly without evidence. Because bacteria are simple morphologically is no proof that they are made up of simple proteins. This

certainly is not true even if it should prove that I have over-estimated the size of these protein molecules.

Tamura working in Kossel's laboratory has made a contribution to the chemistry of bacteria. He used cellular material obtained from the bacillus tuberculosis and mykobacterium lacticola perrugrosum. Both of these were grown in glycerine-broth cultures for five weeks, then collected on filters and washed with ether and alcohol. Tamura states that all the fats and waxes cannot be removed in this way and he resorted to the following method: After partial extraction with ether and alcohol the bacterial cells were rubbed up in a mortar with two parts of sulphuric acid and one of water and from this mass extraction with ether and alcohol was continued. From these extracts Tamura obtained along with the well known fatty acids diamino monophosphatide, a substance which has been previously found in egg-yolk, muscle and brain. Tamura thinks that this body has been mistaken for lecithin by other investigators working with ether and alcohol extracts of bacteria. In these extracts Tamura has furthermore detected a higher alcohol, which he names "mykol" and to which the "acid fast" properties of these bacteria are due. The statement that the fats and waxes including the phosphatide and "mykol" cannot be removed from the tubercle bacillus with alcohol and ether without previous disruption with strong acid is erroneous. In our work, first published in 1908, we showed that prolonged extraction of tubercle cell protein with alcohol and ether removed from the cells the substance to which the "acid-fast" property is due. We extracted first with alcohol in Soxhlets for four days and then with ether for three days. There is therefore no ground for the assumption by Tamura that either the phosphatide or the "mykol" are in chemical combination with the proteins of the cellular substance. It is much more reasonable to regard them as substances either on their way to assimilation into the cell molecules or as excretory products. Tamura says: "My investigations show that the presence of the diamino-phosphatide is not confined to the higher organisms." This is another assumption that the molecular structure of bacterial cells is simple and is wholly without justification. It is additional support of my contention that chemically the bacterial cell is highly complex and should not be regarded as a primitive form of life. Tamura's work on the cellular proteins strongly supports my claim that these are highly complex in chemical structure. He states that he was not able to extract protein from the cell substance with water, salt solution, or one per cent sulphuric acid, and that even with alkali a portion of the cell substance remained undissolved. Surely this would not be true if the cell substance consisted of simple proteins. He also obtained the nuclein bases, diamino and mono-amino-acids. Of the last mentioned his list for the tubercle bacillus contains one (prolin) not found in ours, while ours contains two (glutamic acid and leucin) not found in his. Neither found glycocoll in the tubercle bacillus while we found it in the colon bacillus.

Tamura concludes, as we had done some years before, that bacterial cellular substance contains two carbohydrate groups, but the one which we have designated as chitin-like body, he classifies as a hemicellulose. This name was proposed by Schultze, after an investigation of various cell membranes, to include a group of bodies, "which are wholly soluble on being heated with dilute

alkalis. In the cold five per cent sodium hydroxide dissolves them somewhat more slowly." And yet Tamura classes as a hemicellulose a body which remains in the residue after repeated extraction with one per cent sodium hydroxide; besides he did not test this residue for nitrogen but seems to have assumed that this element was not present since the body did not respond to the protein reactions. In our tests this body remained in the residue after heating for one hour at 120° with ten per cent potassium hydroxide, and did not give the protein reactions, but did contain nitrogen. We know of no nitrogenous carbohydrates except the chitins.

Tamura reports a negative test for sulphur in bacterial proteins, but this is due to faulty technic, since Wheeler has shown that the sulphur is masked and does not respond to the ordinary tests, but its presence is disclosed when a portion of the substance is fused with metallic sodium, dissolved in water and treated with a freshly prepared solution of sodium nitroprussiate; a violet color indicating the presence of sulphur.

While the "acid fast" and "Gram positive" properties of certain bacteria depend upon lipoids which are extracted from the cells by alcohol and ether, the cells, after exhaustive extraction with these solvents, take the analin dyes quite as well or even better than before. For instance the extracted tubercle bacillus stains just as well, or even better, than before extraction with alcohol and ether, but now the stain is easily removed by dilute acid. This behavior of bacterial cellular substance towards basic analin dyes quite naturally suggests that the former consists largely of nuclear material. In my opinion this is strengthened by the studies of the cellular substance which I have outlined. Additional evidence in the same direction is not wanting. When sporogenous bacteria form spores or pass into the resting stage the essential part of the bacterial cell is contained in the spores and all spores and reproductive cells consist in part at least of nuclear material. Certain bacteria which do not form spores pass into a granular state in which potential life continues for a long time. For instance the bacillus of glanders, though an asporogenous organism, may retain viability for a long time. Wladmiroff states that he found these organisms in glycerine-bouillon tubes, with the ordinary cotton plug capable of growth after standing four years. The same is true of the plague bacillus. This phenomenon is explainable only on the assumption that these bacilli contain nuclein. However, assumption is no longer necessary since nuclein, nucleinic acid and their derivatives have been found in all bacterial cells submitted to chemical study. I am strongly of the opinion that the bacterial cellular substance as I have prepared and studied it, freed from the extractives soluble in water, salt solution, alcohol and ether, is practically all nuclear material.

Some years ago A. B. Macallum by microchemical methods showed that nuclei are free from chlorine. His statement is as follows: "Intercellular material and structures, including the so-called cement substance of von Recklinghausen are rich in chlorides but normal nuclei of animal and vegetable cells are absolutely free from them." When my coworkers found no chlorine in the ash of our cellular substance I thought that this must be due to careless work. I could not believe that with the chlorides, especially sodium chloride, as abundantly distributed as they are, they could not be wholly wanting in this material,

but repeated examinations confirmed the first finding. The finding of chlorine would not have been a conclusive evidence that the material is not wholly nuclear, but the failure to find any trace of this element I regard as most convincing evidence that it is wholly nuclear. Furthermore, Macallum found that the phosphorus and iron in nuclear material are masked, that is, they cannot be detected without more or less marked disruption of the molecule. This is true of our cellular substance. I may recall the further fact that the sulphur is so masked that even in the laboratory of so eminent a chemist as Kossel it was not detected in bacterial cellular substance. It is well known that in proteins sulphur exists in two forms, one being readily split off with dilute alkali forming a sulphide, while the other is obtained only when the disruption of the protein molecule is carried much further. The former is wanting and the latter present in our cellular substance. I do not suppose that all nuclear material has the same elemental constituents, indeed, it is not supposable that this is true, but the above facts seem worthy of consideration.

The laborious and valuable researches of Macallum have shown that non-nucleated organisms, such as cyanophyceae, beggiota and yeast cells, contain nuclein, and this is probably true of every cell which is capable of reproduction. We are no longer quite willing to accept the dictum of Schultze, Hertwig and others that every cell must contain a morphologically recognizable part, known as a nucleus. We may insist upon the presence of nuclear matter, but not of nuclei. Some morphologists have seen the necessity of altering our conception of a cell. Bourne has proposed that Schultze's definition be changed to read: "A cell is a corpuscle of protoplasm which contains a specialized element, nuclein."

It should be understood that the cellular substance which I have been discussing is not identical with that which exists in the living multiple bacteria. The latter consists of the former with the addition of all the extractives which I have removed by the solvents, such as water, dilute alcohol, absolute alcohol and ether. The living bacillus has been stripped of all its surrounding food supplies, its accumulated excretory products and its storehouse of fats, waxes, etc. I have a strong suspicion that in some of our bacterial reactions, notably with precipitins and agglutinins, these extractives are concerned, while the cellular constituents have no direct part. The active constituents of the culture, the agglutinable substance, is not, in my opinion, an essential constituent of the bacterial cells, but consists of one or more proteins closely associated with the bacterial cells. It may be a protein already split off from the surrounding pabulum preparatory to absorption and assimilation, or it may be an excretory product. My reasons may be stated as follows: (1) Agglutination does not destroy the viability or virulence of bacteria; therefore, the reaction does not disrupt the living bacterial cell. (2) Thoroughly washed typhoid bacilli are not agglutinable. (3) When typhoid bacilli are thoroughly shaken in salt solution so as to remove their flagellæ and the bacilli are deposited in a centrifuge, the emulsion of flagellæ is agglutinable. (4) Neufeld has shown that when cholera bacilli are thoroughly cleansed by being shaken with one per cent alkali, which does not destroy them and only washes away adherent matter, they are not inagglutinable but produce no agglutinin when injected into animals.

Agglutination and precipitation are closely related phenomena. When a bacterial culture is filtered some of the proteins about the cells pass into solution and constitute the precipitogen while some of the same class of near-cell proteins remain adherent to the cells and constitute the agglutinable substance or the agglutininogen.

I find that the bacterial cellular substances on standing undergo autolytic cleavage. We are just now examining a bottle of colon cellular substance which was prepared ten years ago. It was only air dried and contains a small amount of moisture. When freshly prepared water or salt solution extracted no protein, now one-third the nitrogen passes into solution when the substance is treated with these solvents.

Poisonous Action.—We have found all the bacterial cellular proteins poisonous. Our earlier work was done with the colon cell substance. Since all of these bodies are insoluble in water or salt-solution it has been necessary to administer them in suspension. Early studies demonstrated the following facts: (1) The poison is contained within the bacterial cell and does not under ordinary conditions diffuse into the culture medium.* It is true that old cultures may contain soluble poisons, but these result from autolysis. (2) The poison is not extracted from the cellular substance by water, saline solution, alcohol or ether, either at ordinary temperature or at the boiling point. (3) Heating, even to 140° in the autoclave does not destroy the poison. (4) Dilute (0.5 per cent) solutions of caustic alkali disrupt the cellular substance slowly and imperfectly. Stronger (2 per cent) solutions break up the cell substance and render the poisonous fraction soluble. (5) Boiling with dilute mineral acid (to 1 per cent) has but little effect.

At first we were much puzzled by the fact that smaller doses killed while larger ones failed to do so. This was observed when we were administering the substance by intraperitoneal injection. Then we found that the more finely the substance was ground the smaller was the fatal dose. When the substance was only coarsely ground in a porcelain mortar and suspended in water it did not kill guinea-pigs on intraperitoneal injection in doses less than 1 to 40,000 parts body weight. When the same powder was more finely ground in an agate mortar it killed 15 out of 16 animals at 1 to 75,000; 9 out of 28 at 1 to 100,000; 5 out of 8 at 1 to 200,000; 4 out of 34 at 1 to 2,000,000 body weight. We observed that when heavy suspensions were used lumps of the substance remained undissolved in the peritoneal cavity after death or recovery. In these observations we found the solution of our puzzle. The poisonous action of the cellular substance is in proportion to the extent to which and the rapidity with which, it is split up by the secretions of the body cells and this cleavage is determined by the relative surface exposure of the substance to the action of the cleavage agents. I dare say that the difference in susceptibility as shown among the individual animals is due to the abundance and effectiveness of the secretions elaborated by the body cells.

As has been said, we found the cellular proteins of all the bacteria studied more or less harmful to animals when introduced parenterally. The size of the

*It is understood that we are speaking of cellular poisons and not of bacterial toxins.

dose necessary to produce a fatal result varies greatly with the source of the protein. The cellular substance of bacteria to which in its living state an animal is highly susceptible does not kill that animal at all or does so only after large doses. We have injected into the abdominal cavities of guinea-pigs the cellular proteins of the tubercle bacillus in quantities of from five to two hundred mg. without causing death in a single instance, while on the other hand a fraction of a mg. of the protein from bacillus prodigiosus kills. To kill a guinea-pig one part of the cellular substance of the anthrax bacillus to 1,700 parts of body weight is necessary, while with the colon substance one part to 75,000 kills all animals provided the material is finely ground. In general it may be said that the more highly susceptible a given animal is to infection with a given bacterium the more difficult it is to kill that animal with the cellular protein of that bacterium. On the other hand, the more highly immune a given animal to infection with a given bacterium the more readily does that animal succumb to injections of the cellular proteins of that bacterium. At first sight these statements seem wholly irrational, but when we study them we find that they are not only reasonable but in accord with what might have been reasonably predicted beforehand. The guinea-pig is highly susceptible to infection with the tubercle bacillus because the secretions of its body cells have no destructive action on that organism. This together with the fact that the bacillus tuberculosis can feed upon certain proteins in the guinea-pig's body are the essential factors in the susceptibility. The infecting bacillus finds an abundance of suitable food and meets with no resistance. On the other hand the guinea-pig is highly immune to infection with the bacillus prodigiosus because the animal's body cells supply secretions which are immediately destructive to this organism and the first of these bacilli finding their way into the animal's body meet with immediate and complete annihilation. But when the prodigiosus is grown in vitro and a sufficient amount of its cellular substance, dead or alive, is thrown into the abdominal cavity the same agency which has given the animal immunity to infection now causes it to fall a victim to the protein poison. These facts are of practical as well as scientific interest because they undoubtedly form the basis of the frequently reported and well attested observations of some of the great clinicians of the past that the case mortality in certain infections, most notably in typhus fever, is much higher in the better nourished than in the less robust.

As I have indicated the cellular proteins when introduced parenterally into animals are not wholly harmless even when they do not kill. When the cellular substance of the bacillus tuberculosis is injected into the abdominal cavity of a guinea-pig it has no recognizable effect so far as the behavior or external condition of the animal shows. The dead bacilli are taken up in the folds of omentum and develop local tubercles. When the cellular substance of the colon bacillus is injected, a peritonitis results. In short, the lesions which follow infections result also from the injection of the dead cellular substance. I conclude from this that the lesions of the infections are not due to the activity of the living bacilli, but result from reaction between the bacterial proteins and the body cells.

Split Products.—In 1903, Wheeler and I found that the bacterial cellular proteins could be split into poisonous and nonpoisonous parts and later we showed that all true proteins can be broken up in the same way. This work has been

confirmed by many investigators. There are several ways in which this cleavage can be secured, but the most satisfactory is the one which we first employed. The dried protein, after exhaustive extraction with alcohol and ether, is repeatedly heated at 78° with a two per cent solution of sodium hydroxide in absolute alcohol. When this is done the poisonous fraction goes into solution while the nonpoisonous part remains undissolved and is removed by filtration. This is evidently a true cleavage and not a mere disintegration. The nonpoisonous portion contains all the carbohydrate and phosphorus of the original complex molecule.

The Protein Poison.—Since this body has been obtained from all true proteins, bacterial, vegetable and animal, so far examined, we have called it “the crude soluble poison;” “crude” because it is undoubtedly a mixture of chemical bodies and “soluble” in contradistinction to the bacterial cellular proteins from which it was first prepared. Aqueous solutions are somewhat opalescent, and may be quite turbid. Filtration through hard paper generally gives a clear filtrate but with some preparations we have found filtration through porcelain necessary to secure a perfectly clear solution. All the crude soluble poisons that we have obtained give the biuret and Millon tests. None give the Molisch test, thus showing the absence of carbohydrate. Some give the Adamkiewicz and Liebermann tests while others do not. This test is believed to be due to the presence of tryptophane. The fact that the poisons from certain proteins do not respond to these tests indicates that Doerr’s assumption that the poisonous action is due to the presence of this group is without support. The poison gives the Millon test most strikingly and in high dilution. This test is believed to indicate the presence of tyrosine and it is interesting to note that gelatine which contains no tyrosine does not yield the poison. Aqueous solutions are distinctly acid to litmus and this reaction is due to some organic body. Neutralization with alkalis and alkaline earths weaken the action of the poisons. Poisons from some proteins appear to form definite compounds with calcium and magnesium and at least some of the calcium bodies are inert. In the dry state the protein poison forms a brownish powder varying somewhat in shade with the protein from which it is obtained. All preparations have the same marked odor. It is much more freely soluble in absolute alcohol than in water. Whether it should be called a protein or not is a question. Proteins should not be soluble in absolute alcohol. However this substance gives the biuret test and this is generally regarded as the most distinctive test for proteins. Its alcoholic solutions are precipitated by alcoholic solutions of copper, mercury and platinum. By means of these precipitants with subsequent removal of the metal with hydrogen sulphide, we have obtained our most potent preparations. By this method we have obtained a body which kills guinea-pigs of from two hundred to three hundred grams weight in doses of 0.5 mg. given intravenously. The poison is not an alkaloid, although it may be basic in character.

Action on Animals.—The comparative effects of the living bacillus, the dead cellular substance and the crude soluble poison on animals was first worked out by V. C. Vaughan, Jr. The organism used was the colon bacillus.

(a) *The Living Bacillus.*—When a guinea-pig receives a fatal dose of the living colon bacillus intraperitoneally there is a period of from five to twelve hours, varying with the size of the inoculation, during which there are no recog-

nizable symptoms. We regard this as the period of incubation, and it is roughly proportional to the amount of the culture used and to some extent to the virulence of the organism or the rate at which the bacillus multiplies. This work was done with a bacillus, 1 c.c. of a twelve hour or older bouillon culture of which invariably killed within twenty-four hours. When this amount was given no effects became visible for a period of from ten to twelve hours. With larger doses the period of incubation was somewhat shorter, but with the largest doses of the richest cultures there is still a period of incubation. This measures the time necessary for two things to happen. First the bacillus must multiply sufficiently to supply enough poison to visibly affect the animal. Second, this poison must be made effective by being split out of the large molecule of which it is a part. Therefore, while the period of incubation is not accompanied by the development of symptoms which rise to the plane of observation, it is actually a critical period in every infection and the outcome depends upon whether the bacteria are all destroyed before a lethal dose of the poison has been developed by the multiplication of the bacillus and set free or made effective by the secretions of the body cells. It is during this period that natural and acquired immunity either save the day or, for the time at least, fail. In natural infection the number of bacilli introduced is small and in case of full immunity these are all destroyed, there is no multiplication and the amount of poison set free in the destruction of the small number of the invaders is not sufficient to induce symptoms or to develop lesions. This is what happens when the smallpox virus finds its way into the body of one thoroughly immunized by a previous attack of the disease or by successful vaccination. When the immunity is only partial or when the infection is massive or unusually virulent, the virus develops for a time, becomes more or less distributed in certain tissues and its final destruction is accompanied by the development of symptoms, and the reaction between the virus and the body cells leaves more or less marked lesions. When there is no immunity the virus multiplies without hindrance and life is destroyed. There are infections in which the body shows little or no resistance. Some of these run an acute course and destroy life in a few days, while others are more chronic. This seems to depend upon the rate of multiplication in the invading organism. Apparently there is relatively as much difference in the rate of multiplication in bacteria as there is among the higher animals. The "generation period" or the interval between fissions varies among species and strains, and is influenced by external conditions. Virulence is largely determined by rate of multiplication or at least the two correspond. Under favorable conditions the cholera bacillus divides about every half hour. So far as I know no one has determined the "generation period" in the tubercle bacillus, but it is certainly much longer. It follows that cholera is an acute disease, often terminating fatally in a few hours, while tuberculosis extends through months and even years. The guinea-pig shows no resistance to the tubercle bacillus and the organism slowly but steadily grows, develops its characteristic lesions and kills, probably through its autolytic products and without developing any antagonistic action in the body cells. Rodents, especially rats, show but little or no resistance to the plague bacillus, except in those regions where this disease is endemic and there, it is said, this disease even among the rats becomes a chronic infection.

Our intraperitoneal infection of the guinea-pig is comparable with the development of a general peritonitis from a ruptured appendix. The period of incubation is short and while there may be some elevation of temperature, this is not marked or even constant. During the period of incubation, when the bacilli are abundantly multiplying, the behavior of the animal in no way distinguishes it from its untreated fellows, but at the end of this period there is a marked change. The animal no longer eats; its coat becomes rough; its head droops; it sits in one corner of the cage in a stupor; its abdominal walls become rigid and pressure over this region elicits evidence of pain. Now, its temperature begins to fall and this decline is progressive in fatal cases. We have frequently seen the temperature fall from 101° to 94° in from two to four hours and it may reach 85° and even lower before death. A rise in temperature after it begins to fall generally means recovery. Autopsy reveals a general hemorrhagic peritonitis with a large amount of bloody fluid with intact red corpuscles and leucocytes in the peritoneal cavity. The parietal and visceral peritoneum are studded with minute punctiform hemorrhages and there is more abundant hemorrhage in the great omentum. The chemotactic pull of the bacilli has been not only great enough to assemble great numbers of leucocytes, but violent enough to rupture small blood vessels.

(b) *The Cellular Proteins.*—When a fatal quantity of the cellular protein of the colon bacillus is injected into the peritoneal cavity of a guinea-pig the progress of events is exactly like that following infection with the living organism except that the period of incubation is shortened. There is no longer either opportunity or need for the multiplication of the bacillus. This has taken place in vitro and enough of the protein to kill has been introduced. One of the features that characterize and mark the period of incubation has been withdrawn. It only remains for the body cells by means of their secretions to cleave the bacterial protein and set the poison free. The period of incubation is reduced half or more, then the evidences of poisonous action are exactly the same as in the inoculated animal. The temperature falls at the same rate and autopsy reveals exactly the same lesions. The chemotactic pull of the dead protein has proven just as strong and just as violent as that of the living protein. In fact the pull in both instances is a chemical and not a vital one and the lesions result from a reaction between the proteins of the bacterial cells and those of the body cells.

(c) *The Soluble Poison.*—When a fatal dose of the crude soluble poison is injected into the peritoneal cavity, the effects begin to reveal themselves much sooner. There is now no period of incubation. Both steps, which have characterized this period, are now omitted. The bacillus has been grown and has been cleaved in vitro. The action of the poison begins to manifest itself within a few minutes—from five to twenty—and it appears in three, well marked stages: The first we have designated as that of peripheral irritation. In the guinea-pig it is manifest by the animal scratching itself, generally first on the nose and then over every part of the body which can be reached by its claws. In man an erythematous blush, beginning about the point of injection, spreads over the body and may be followed by an urticarial rash with intense itching. This is not always confined to the cutaneous surface, but may extend to the mucous membrane of the mouth, throat and rectum. The second stage is one of partial paralysis.

The guinea-pig lies on its side, with rapid, shallow and difficult breathing. When urged to move it shows inability to coordinate its movements and partial paralysis is evident, especially in the posterior extremities. In man the breathing becomes distressingly asthmatic. Air-hunger is marked and there is a sense of impending danger. The convulsive stage marks the termination. The convulsions are usually clonic and at first generally involve only the neck muscles, the head being thrown back. The seizures extend over the body, becoming more frequent and violent. During a convulsion, occasionally in an interval, respiration ceases. The heart continues to beat, at first with no acceleration and with perfect regularity. The exact mode differs somewhat in different animals, but is always that of anaphylactic shock. Necropsy shows the same conditions found after death from anaphylactic shock. The peritonitis found after death from inoculation or from the injection of the unbroken cellular protein is wholly wanting.

When a nonfatal dose of the soluble poison is administered, the symptoms are those described above as characterizing the first and second stages. There may be isolated and slight convulsive seizures, but an animal seldom recovers after the convulsions have become general and frequent. With recovery the temperature slowly rises and ultimately returns to normal. Within two hours the animal is apparently quite normal in every respect.

(To be continued.)

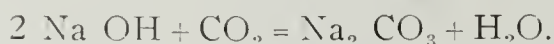
THE PHARMACOLOGICAL ACTION OF NITROUS OXIDE*

BY D. E. JACKSON, PH.D., M.D., ST. LOUIS, MO.

IN a previous article¹ I have described a closed method for the administration of nitrous oxide and other anesthetics in conjunction with oxygen. In the present instance I want to discuss certain features of the pharmacological action of nitrous oxide as studied by this method. No morphine, scopolamine or other hypnotic has been used in any of these experiments.

I may refer briefly to an improved form of apparatus which I have used in these experiments (Fig. 1). A rotary pump of less capacity than that used in the device previously described is attached to a very much smaller and more compact frame. The chief object in this has been to simplify the apparatus and to reduce its size and weight. A number of valves, tubes, etc., shown in the illustration have been found by experience to be unnecessary, but in the experimental development of the device they were included as precautionary measures. The apparatus carries only two tanks, one for oxygen and one for nitrous oxide, for experience has shown that since tanks need be renewed only at considerable intervals, and, if the breathing bag be filled moderately full at the moment when either tank becomes exhausted there will be ample time to remove the empty tank and replace it by a new one before a fresh supply of the gas (generally oxygen, of course) is required. Realizing the great value of simplicity and lightness in any form of apparatus intended for constant use, I have spent much time and energy in trying to produce as simple a device as possible. It is perfectly evident that the apparatus here shown is much more complicated than it need be, but for the benefit of others who may be interested in the subject of nitrous oxide anesthesia, I have thought it worth while to include here an illustration of the apparatus with which much of the work discussed below has been carried out. For a description of the general principles on which the device is operated I must refer the reader to the article indicated above. I may state briefly, however, that by means of a motor and a rotary air pump, air or other gaseous or volatile substances (chiefly nitrous oxide and oxygen so far as the present article is concerned) are kept circulating within a closed system of tubes and vessels, and through a breathing bag into and out of which the animal breathes.

The vessels are two in number and consist of glass jars, the one containing sulphuric acid which serves to sterilize, dry and warm the air (or gases) which are washed through the acid, while the other jar contains sodium hydrate solution through which the air or gases, including the exhaled CO₂ from the patient are washed. The CO₂ is immediately absorbed by the sodium hydrate forming sodium carbonate and water according to the following equation:



The sodium carbonate being a soluble salt of course remains in solution (to-

*From the Department of Pharmacology of Washington University Medical School, St. Louis, Mo.

gether with the H_2O formed) in the jar, the CO_2 being thus removed from the air (or nitrous oxide and oxygen) which the animal breathes. During this process the oxygen is consumed (250 to 300 c.c. per minute for an adult man at rest) by the animal or patient. More oxygen is injected into the system from time to time in just such quantities as the animal actually consumes. The nitrous oxide, being a stable gas, is not broken down at all either by the animal or by the acid or sodium hydrate. Consequently there need be but little waste of the

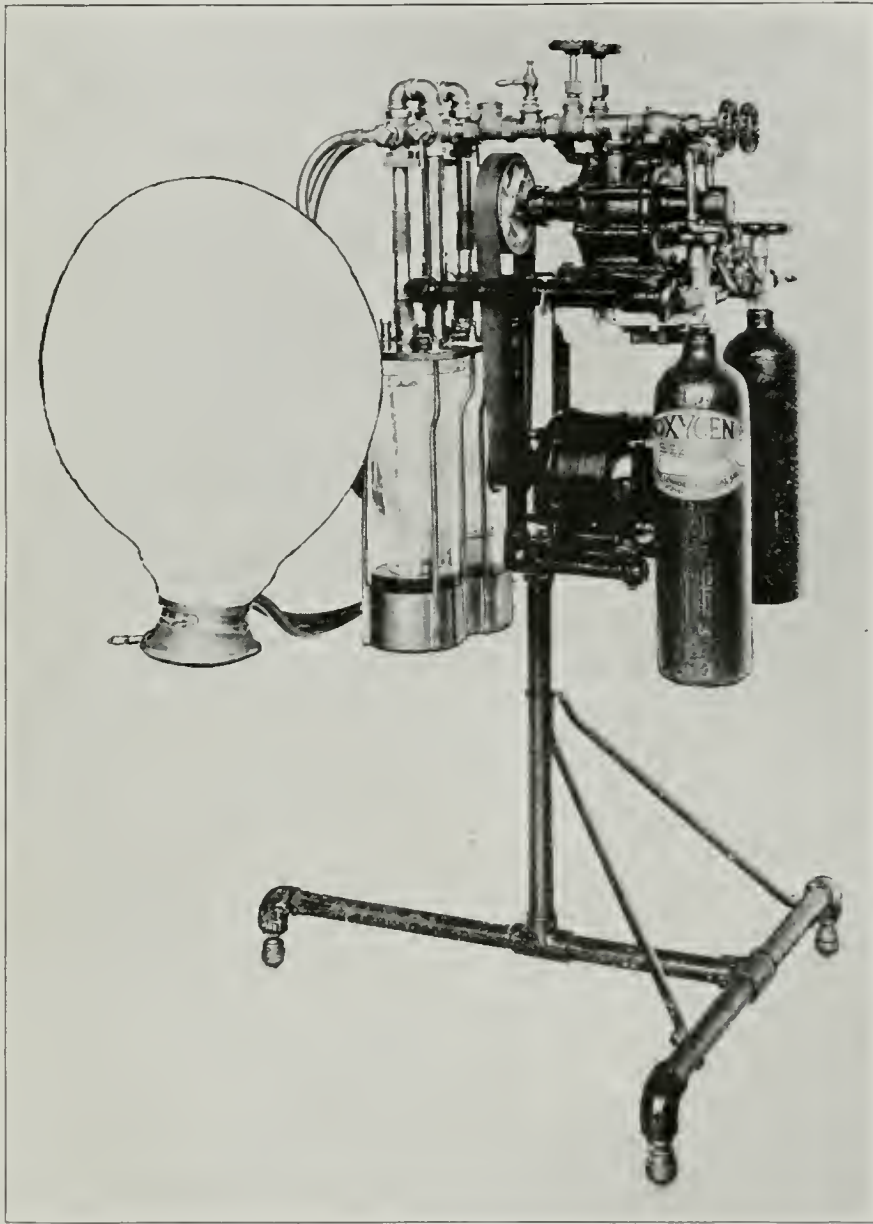


Fig. 1.—Nitrous oxide apparatus with large bag as used for experimental purposes. (For discussion, see text.)

N_2O and only a small amount (experimentally I have estimated that from $1\frac{1}{2}$ to 3 gallons will be necessary for a man weighing 160 pounds) is required to saturate the blood sufficiently to produce anesthesia. When a given amount of N_2O is injected into the closed system and breathing bag, the animal, whose lungs virtually form a part of the closed system, will at once begin to absorb N_2O into its blood from the pulmonary alveolar walls. This absorption goes on until an equilibrium in the quantity of N_2O contained in the animal's blood and tissues on the one side and that contained in the breathing bag and tubes on the other

is established. It is important, however, to remember in this connection that the affinity of the blood and presumably of the central nervous system is greater for N_2O than is the affinity of water for N_2O . So far as is known at present N_2O does not form any special chemical combination with the blood or other tissues of the body. It is apparently held in solution in the blood and tissues in the same way as any other indifferent gas dissolves in a liquid, i.e., in direct proportion to the partial pressure exerted by the gas on the liquid. The lipid content of the blood and central nervous system is generally considered to account for the increased solubility of the gas in these tissues over that in water.

The breathing bag and face-piece shown in Fig. 1 are also modified considerably from that which I first used. The bag shown here I have found well adapted for experimental observations. It is chiefly from this standpoint that I want to discuss the action of nitrous oxide in this article. This bag holds three gallons, but the sides of the bag are flat and when not in use fall together as the air or gas passes out. When in use only about one or two gallons of air or gas need be injected into the bag and this permits the subject to have a full and free opportunity to breathe in any way he pleases. It is desirable that no excess pressure, as from an overfilled bag, be introduced to embarrass the breathing of the subject. The excess amount of work which may thus be easily thrown on the respiratory apparatus in the course of an hour may be astounding, as a brief mathematical calculation will readily show. And the problem is still further complicated both directly and indirectly by the embarrassment to the heart and lung circulation which the amount and peculiar application of this excess work involves. A further feature to be noted in the face-piece is the large opening, about three and one-half inches in diameter, which connects the bag to the air cushion resting on the face. Thus the subject breathes almost directly into the large flexible bag and obstruction of the respiration is reduced to a minimum. While this bag and face-piece serve very well for experimental observations, there are certain objections which may be made to them from a practical standpoint. The first of these is the difficulty of making an air-tight contact between the subject's face and the rubber cushion on the face-piece. The second is the inconvenience of having the large bag near the patient's head. At present, however, I wish to avoid any extensive discussion of the clinical side of this subject.

THE GASES INVOLVED IN NITROUS OXIDE ANESTHESIA.

The pharmacological relations of at least four gases must always be considered in nitrous oxide anesthesia. These are N_2O , oxygen, CO_2 and nitrogen. And it may be worth while to remember that a small amount of argon, neon, crypton, xenon, etc., are also present. Ordinarily these gases are supposed to be inactive in the animal organism, but under the peculiar conditions established in the gaseous content of the body under nitrous oxide anesthesia, I am inclined to believe that the presence of these substances, at least at the beginning of the anesthesia, should not be entirely forgotten.

It should be emphasized that the method which I have here used permits investigation of peculiar gaseous relationships which no other device heretofore employed for this purpose could well reveal. For by this method the supply of the four gases (N_2O , CO_2 , N, and O) concerned in the animal's respiration,

may be separately and independently controlled. The CO_2 , of course, is eliminated by the animal, but it may be allowed to accumulate in the breathing bag for experimental purposes and its relative action in combination with the other gases thus studied. The relative effects of CO_2 and O in ordinary forms of breathing and in asphyxia have been thoroughly studied by numerous investigators.² When, however, nitrous oxide is introduced, the conditions are very materially changed and only a small amount of work has been done on this phase of the problem.

It was supposed by Sir Humphrey Davy that nitrous oxide was decomposed in the body which thus became flooded with an excess of oxygen which was promptly changed to carbon dioxide. This carbon dioxide then acted as a depressant and caused the anesthesia. It was later shown that nitrous oxide was not thus broken down but was excreted by the lungs in the same form as that in which it had been absorbed. The theory then became prevalent that nitrous oxide acted solely by excluding oxygen from the tissues and that its action was chiefly a matter of asphyxia. That asphyxia may, and in practice certainly does often play a considerable part during the production of the anesthesia, no one at present doubts. But it has been thoroughly established that nitrous oxide possesses distinct specific depressant powers of its own on the central nervous system. In 1897 Kemp³ published a series of observations on the gaseous content of the blood during nitrous oxide anesthesia. He drew off blood from the femoral artery of dogs anesthetized with various mixtures of N_2O and air and of N_2O and O, and found that complete anesthesia could be produced by the gas when the blood contained quantities of oxygen fully capable of maintaining consciousness and of carrying on the ordinary process of metabolism. When nitrogen was substituted for the N_2O , the percentage of oxygen breathed remaining the same, then the anesthesia gradually passed off and the animal regained consciousness. And it has been found by the late Sir Frederic W. Hewitt⁴ that a mixture of nitrous oxide 80 per cent and oxygen 20 per cent (the amount present in air) is fully capable of producing anesthesia in suitable subjects. These observations prove beyond doubt that N_2O possesses specific depressant powers on the central nervous system. It has also been shown by Kemp as well as by others that under N_2O anesthesia the CO_2 content of the blood is greatly reduced below the normal. But in most cases, however, it has been found that the oxygen content of the blood is reduced in even still greater degree below the normal than is the carbon dioxide. As ordinarily administered N_2O causes the nitrogen (and presumably the argon, etc.) contained normally in solution in the blood and tissues to be rapidly washed out of the system. Kemp's blood analyses for the dog show in several experiments a complete absence of nitrogen from the gases drawn off by the vacuum pump. It is to be noted that in all other forms of anesthesia the nitrogen (about 1.7 vol. per cent) remains dissolved in the blood. *Does the absence of this supposedly inactive gas in any way affect the anesthesia?* In many instances I have observed dogs going under the influence of N_2O in which it appeared to me very probable that the elimination of this nitrogen was essential to the production of successful nitrous oxide anesthesia. It is, unfortunately, extremely difficult to prove this point. For one must, as a general rule, empty out most of the air (nitrogen) from the apparatus (and lungs and tissues

of the animal) in order to fill this space with nitrous oxide so as to be able to obtain a sufficiently high percentage of the gas to produce the anesthesia. This makes difficult the solution of the question as to whether or not the absence of the nitrogen in any way influences the nature of the anesthesia.

In most forms of nitrous oxide apparatus used heretofore breathing had to be carried out under a greater or less degree of pressure. It is interesting to consider what influence, if any, this may have in tending to dam back the CO_2 produced in the tissues. While this gas did not apparently accumulate in large quantities in the blood in the analyses made by Kemp, still one is inclined to suspect that the tissues may have been trying to form the ordinary amounts of the gas but were either unable to do so or else they could not pass it over to the blood. And any such accumulation of CO_2 in the tissues may very well influence the nature of the anesthesia produced. And similarly any of the immediate precursors of CO_2 , if allowed to accumulate in the tissues or blood, may affect the character of the anesthesia produced.

THE SYMPTOMS PRODUCED BY NITROUS OXIDE.

I have studied this topic both from the standpoint of animals and from that of man.

A frog placed in an atmosphere containing a high percentage (90% to 98%) of N_2O becomes well anesthetized in from three to four minutes. When again placed in fresh air the animal fully recovers in about one minute. Profound anesthesia is readily obtained.

The symptoms in dogs vary greatly with the animal and the method of administration. Fig. 2 shows a record of the respiratory movements in a dog just beginning to inhale N_2O . The animal was lying quietly on the table and made no resistance in any way. The record was obtained by tying a stethograph around the chest wall and connecting it by rubber tubing to a recording tambour. The first part of the tracing shows the normal respiratory movements when the animal was breathing a sufficient percentage of oxygen and the CO_2 was not allowed to accumulate to excess. At the point indicated N_2O was run into the breathing bag and shortly thereafter the depth of the respiration began to increase. This is mainly due to the action of N_2O . It is the typical effect of this gas on the respiration. There is one other point to be considered in the experiment, however, and that is the fact that in this case when the N_2O was run into the bag then the oxygen which the bag contained was considerably diluted. This would also cause the animal to breathe more deeply. But independently of this dilution of the oxygen, the first effects of nitrous oxide in sufficient concentration appear to be to stimulate the respiratory center. After a time, as the animal passes more fully under the influence of the gas, the depth of the respiration decreases while the rate varies somewhat, but on the whole is accelerated beyond the normal. This does not seem to be due to any accumulation of CO_2 in the breathing bag or apparatus, for it is very easy to wash out the CO_2 as fast as it is formed. The animal usually does better, however, if a certain amount of rebreathing and CO_2 accumulation is permitted.

These same phenomena occur in the human subject. It is very interesting to experience the beginning action of the gas. If one fills the bag partly full of

oxygen and breathes this for a while (washing out the CO_2), he may at first note a very slight sense of fullness in the head and possibly there may be a feeble flushing of the skin especially of the face and neck. Whether this is due to a slight CO_2 accumulation in the lungs (dead space, etc.), caused by the small amount of obstruction to the normal respiration, or is due entirely to excitement or the mere feeling that one "expects something" I have not been able to determine. It is of but little consequence, however, and soon passes off as one adjusts himself to breathing into and out of the bag. Slight odors, as from a new rubber bag, or of oil from the pump, etc., sometimes cause one to be a little apprehensive. And the mere act of fixing the attention on the respiration is sufficient to cause certain minor variations in most subjects. When the N_2O is turned on, however, there is an immediate feeling of ease in breathing. The sensation can best be compared to the effect of oiling a new machine. One is somewhat surprised how readily he can breathe deeply and fully and without special exertion. This sensation does not occur if one instead of running nitrous oxide into the bag, should fill it to a corresponding degree with oxygen. I have been inclined to believe, therefore, that it is due to a direct stimulation of the respiratory center by the N_2O . I have considered the question of whether or not the processes of diffusion of the gases in the lungs, or the rate or ease of absorption or excretion of the oxygen or CO_2 through the alveolar epithelium might be influenced in any way by the presence of N_2O rather than of nitrogen. I have not been able to reach any conclusion on these matters. It seems probable that certain obscure changes are produced in the metabolism of the tissues on account of the markedly subnormal CO_2 and oxygen content of the blood, as shown by Kemp. It would be interesting to know whether or not these low percentages of CO_2 and oxygen persist in the blood in those cases in which anesthesia is produced by approximately 80 per cent N_2O and 20 per cent oxygen as in Hewitt's experiments. I have occasionally believed that in rare instances in dogs which were fully anesthetized I could raise the percentage of oxygen in the bag to perhaps 30 per cent without allowing the animal to revive. In this case, of course, while I might markedly increase the percentage of oxygen in the bag, I did not correspondingly lessen the amount of N_2O in the animal and in the apparatus. In this feature there is a great difference between the apparatus which I have here used and most other forms of nitrous oxide machines, for in these if the amount of oxygen administered is increased, this generally means a corresponding diminution of the amount of N_2O given with correspondingly increased chances for variations in the character of the anesthesia.

I have noted only occasionally, as have a number of my students, that just as one begins to breathe a fairly concentrated mixture of N_2O , there may be detected a faint metallic sweetish taste on the tip of the tongue. The sensation reminds one of the taste of saccharine. In my own case this taste has never lasted for more than a second or two, but one student was able to detect it over a prolonged period. It is probably due to N_2O carried in the blood from the lungs to the taste organs.

It will be noted from Fig. 2 that the animal did not struggle as the gas was administered. In some cases I have seen dogs go quietly to sleep and apparently never be conscious at all that they were being anesthetized. In a gen-

the animal which is especially susceptible to the gas, this may frequently occur. It is by no means the rule, however, and there is often struggling especially if the animal was excited before the anesthesia was started. It is well known that certain human subjects are especially resistant to the gas and I have frequently found this to be true for dogs. In some cases I have been entirely unable to obtain any true anesthesia at all. In these cases cardiac slowing and other complications nearly always come on as one attempts to crowd the gas. This appears to be partly due to stimulation of the vagus center in the medulla for section of the vagi usually accelerates the heart and this is generally even more marked after atropine. I have seen three or four especially striking cases of

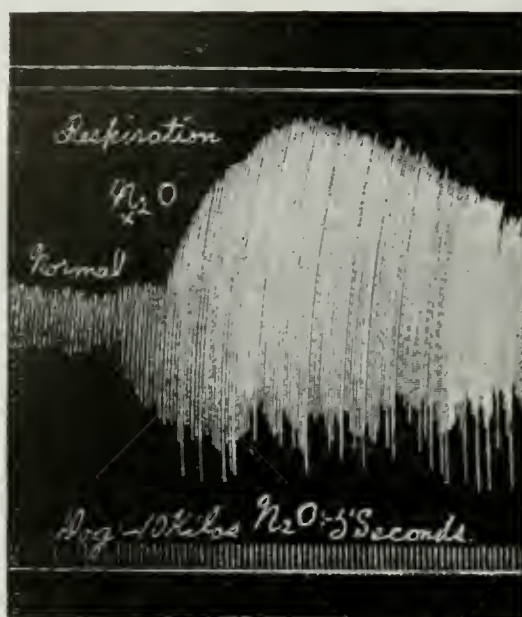


Fig. 2.—This tracing shows the respiratory movements of a dog to which (at X) nitrous oxide (plus oxygen) was administered. There is a slight exaggeration of the respiration because the animal had been breathing mainly with its diaphragm before the gas was given, but after this the chest movements became much increased. The stethograph recorded more of the thoracic movements than it did of the abdominal.

this kind. An animal which is excited or struggles is much more liable to manifest these cardiac symptoms. After atropine the animal usually takes the gas considerably better, indicating better aeration of the blood in the lungs from the improved circulation. The slowing of the heart may be very marked and apparently may be the cause of death in some cases. I have not taken string galvanometer tracings of the hearts of these animals, but this would be instructive. It has seemed to me in one or two instances that peculiar arrhythmical contractions were set up in the heart and that this finally ended suddenly either with complete stoppage of the heart, or with the establishment of a condition resembling heart block. The cause of these reactions is not at all clear. One would suspect a lack of oxygen, or CO_2 poisoning, but when the CO_2 is well washed out of the gases breathed and the conditions are the same as those under which other animals have been well anesthetized, then one is inclined to look for a difference in the animals. I suspect that very nearly, if not quite this same thing, may have occurred in a few instances in man.⁵ For that reason I wish to refer briefly to Fig. 3 which is a tracing of the apex beat of a dog which was given nitrous oxide. The animal was not a good subject, but was finally apparently well anesthetized. After the anesthesia had continued for perhaps half an hour,

the pulse in the femoral artery became irregular and finally stopped rather suddenly. With considerable difficulty the animal was revived by means of intermittent compression of the chest. But when the respiration was restored, the animal did not promptly regain consciousness and remained in a semicomatose or somnolent condition for two or three hours. It was noticed about five hours after the animal revived that the heart was irregular and the tracing here shown was made. The animal improved and in about a week the heart had apparently returned to normal. The animal was kept for forty days thereafter, but no further cardiac disturbance was observed.

It seems evident to me that the human subject must be very much more susceptible to nitrous oxide than is the average dog. The anesthesia in all cases is of a much lighter form than that produced by ether. In dogs it is as a rule impossible to destroy the corneal reflex, for in the deepest anesthesia in these animals the slightest touch of the cornea or eye lid or even eye lashes causes immediate winking. The eyes remain open and keep up peculiar rolling or staring movements so that one often wonders whether or not the animal is fully anesthetized. If the gas be removed suddenly, however, the animal wakes up and

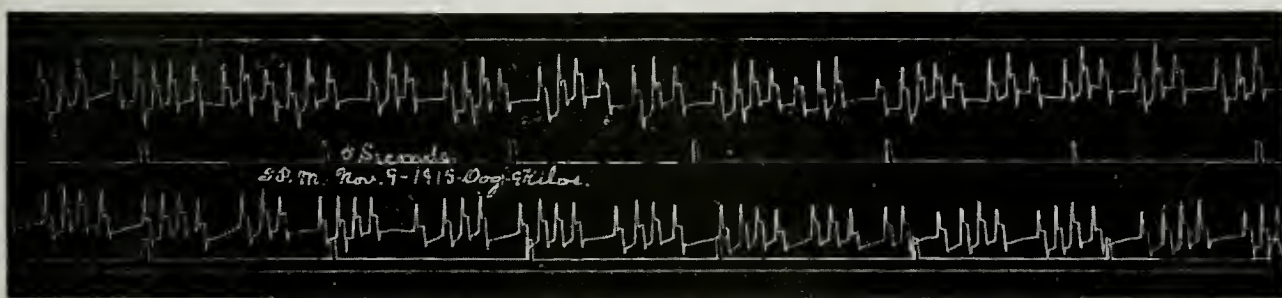


Fig. 3.—Tracings of the apex of the heart in a dog in which under nitrous oxide anesthesia cardiac irregularities developed. For a full description see text. This tracing was taken about five hours after the animal had been anesthetized. At this time it was observed by Mr. John A. Higgins that the animal was in a dazed or semicomatose condition, and that the heart beat was very abnormal. The record here shown was then made.

stares about in a way which shows that it had been completely unconscious. During the anesthesia the pupils are dilated but the light reflex is preserved.

THE ACTION OF NITROUS OXIDE UNDER VARYING CONDITIONS.

If pure N_2O be inhaled, unconsciousness results in a period of from thirty to sixty seconds. But if oxygen be added to the inhaled gas, the time required to produce unconsciousness rapidly increases as the oxygen rises from zero up to five, ten or more per cent. With more than ten or twelve per cent oxygen content mixtures of nitrous oxide and oxygen usually produce unconsciousness only after considerable periods or not at all, depending on the patient. There seem to be great variations in this respect, however, in the human subject, and I have noted a similar reaction in animals. I should like to emphasize this point in particular since it has a direct bearing on the administration of nitrous oxide by the method which I have here used.

It will be noted by reference to Fig. 1 that there is a considerable "dead space" in the apparatus. The wash jars, tubes, pump, etc., and the breathing bag all represent space which in the beginning contains air. When the animal is connected to the apparatus then its lungs also add "dead space" to the system.

This "dead space" contains oxygen and nitrogen. The oxygen can be readily used up by the animal, but the nitrogen must be gotten rid of. The amount of this nitrogen depends on the construction, size, etc., of the apparatus and on the size of the animal. The blood (and tissues) of the animal also contain about 1.7 per cent of nitrogen which presumably diffuses out into the lungs and is then breathed out when nitrous oxide and oxygen are administered. It is necessary to remove a large part of this nitrogen from the apparatus to secure the best success. This is done by filling the bag partly full of nitrous oxide and running the pump for a while. The animal breathes the mixture of air and N_2O and absorbs a portion of the gas, while at the same time some of the nitrogen in its blood is breathed out. The sodium solution and the sulphuric acid in the wash jars also absorb some nitrous oxide. In a little while the bag is emptied out into the air. This is accomplished by opening a valve on the right (positive) side of the machine while the pump is running. In one or two seconds the bag can be emptied as much as desired and then more N_2O is run in until the bag is about one-half or two-thirds full. This is repeated about three or four times as a rule with dogs. One should not hurry this process. It usually takes at least five minutes to anesthetize a dog deeply and any attempt to crowd the gas faster generally excites the animal and does not improve the anesthesia. It is better to proceed slowly and allow the animal's blood to become as nearly saturated as possible for each concentration of the N_2O . In this manner the action of the drug is brought on slowly and in a perfectly successful experiment the animal may be fully anesthetized apparently without being conscious that anything unusual is occurring. Often it is not necessary to give any oxygen until the animal is anesthetized, for the oxygen in the apparatus and in the lungs, etc. ("dead spaces") serves to keep the animal in good condition for some time. When needed, however, more oxygen should be injected.

It may seem that five minutes is an unreasonably long time to require for the production of nitrous oxide anesthesia. We should remember, however, that much more time than this may be required with ether, etc., and when we think of anesthetizing an animal in thirty to fifty seconds with nitrous oxide it is interesting to consider the possibility of doing this same thing with chloroform or ether vapor which are exceedingly well absorbed by the blood. And it is probable that in any rapidly produced nitrous oxide anesthesia there may be a considerable element of asphyxia which is undesirable.

In this connection I should like to refer to some physiological experiments⁶ on respiration which involve certain features usually concerned in nitrous oxide anesthesia.

"1. *The Immediate Effects of Total Rebreathing (Due Chiefly to Excess Carbon Dioxide).*—The nostrils are compressed with a nose-clip and the subject breathes from and into a rubber bag containing 20 to 40 liters of air. The amplitude of respiration is soon augmented, and in the course of a few minutes the subject is panting heavily forty times a minute. He usually develops a typical carbon dioxide headache, but this wears off in fifteen or twenty minutes after the experiment is ended." These results are produced by breathing for a "*few minutes*" into a closed bag. If in addition to these effects, which are due chiefly to carbon dioxide accumulation, there be added the further effects of oxygen

want which are usually present from the very beginning in the administration of nitrous oxide, what will be the results of these purely physiological phenomena when complicated by the addition of nitrous oxide in those forms of apparatus in which rebreathing into and out of a closed bag is carried on for considerable periods of time?

"2. *The Effects of Insufficient Oxygen Without Excess of Carbon Dioxide.*

—The above mentioned bag is refilled with 20 to 40 liters of fresh air and the experiment performed again, but with this difference, that a vessel of 1 or 2 liters capacity filled with soda-lime or broken sticks of sodium hydrate is placed between the bag and the subject's mouth so that he breathes through it into and from the bag. The carbon dioxide exhaled by the subject is thus absorbed, and he gradually consumes the oxygen in the bag. As a rule there is *no noticeable deepening or quickening of the breathing*, and the subject will first become cyanosed and then unconscious without appreciable augmentation of breathing. This experiment should *always be carefully supervised*, as it is not free from danger. If continued for more than ten minutes, it is usually followed by a severe frontal headache, developing slowly for several hours thereafter, together with other ill effects and lasting from twenty-four to forty-eight hours." It is particularly interesting to consider this experiment in connection with those forms of nitrous oxide apparatus in which the patient inhales the gas (plus a varying but usually small amount of oxygen) from a tank or reservoir and then exhales out into the open air. In these machines the carbon dioxide is probably fairly completely removed as fast as it is exhaled from the lungs. The small percentage of oxygen usually given (e.g., from two to ten or twelve per cent) with the nitrous oxide may cause a rather close simulation of the conditions established in the above experiment in which *cyanosis* and *unconsciousness* may be produced *without any anesthetic*. I should like to give one further quotation bearing on this point from Haldane and Poulton.⁷ * * * "Still more sudden exposures to anoxemia occur when air containing little or no oxygen is breathed; for in this case the oxygen previously present in the alveolar air, and even in the venous blood, is rapidly washed out; the result is that consciousness is suddenly lost *without evident preceding hyperpnea*, although abundance of CO₂ is present in the arterial blood. Haldane and Lorrain Smith observed sudden loss of consciousness after 50 seconds on breathing air which was afterwards found to contain 1.8 per cent of oxygen. During any exertion the loss of consciousness is still more sudden. Thus it is a common experience with miners going into an atmosphere of nearly pure fire damp (CH₄), or climbing up so that their heads are in the gas, that they drop suddenly as if they were shot."

I do not care to discuss this point further, but may state briefly that my own experiments, together with the results obtained by others, have led me to conclude that it is impossible to obtain a rapid (1 minute) production of anesthesia and unconsciousness in dogs with nitrous oxide and oxygen at atmospheric pressure unless the oxygen content of the mixture is so low that the loss of consciousness is due *almost entirely* to the lack of oxygen. Presumably, with certain modifications, this is true in the human subject also. On the other hand it seems probable that in all dogs which do not possess a special idiosyncrasy against the gas, mixtures of nitrous oxide and oxygen containing sufficient amounts



Fig. 4.—Blood pressure and respiration in a dog anesthetized with ethyl chloride. At three places, as shown on the record, nitrous oxide was run into the breathing bag. This was done in order to observe the effects of the gas on the circulation and respiration. The results were practically nil so far as can be observed from the record.

of the latter to avoid most if not all asphyxial effects, may be used to produce anesthesia *provided sufficient time be allowed for the gas to act and the CO_2 be completely removed as fast as it is excreted by the lungs*. If a high per cent of oxygen is used, anesthesia cannot be quickly produced but asphyxia may be avoided. The time required may be considerable, perhaps from five to fifteen minutes or longer. But as the tissues gradually become more and more saturated with the gas, there will be a gradual depression of the central nervous system which will finally result in unconsciousness.

It was long ago observed by Goldstein^s that anesthesia appears more quickly and with a proportionately less degree of asphyxia, the higher the organization of the brain—namely, earlier in man than in laboratory animals. I have been able to confirm this observation many times. And in addition the anesthesia appears as a general rule to be deeper in man than in dogs, although in some ani-



Fig. 5.—This animal was anesthetized with nitrous oxide. At the point indicated ethyl chloride was injected into the bag. There is an immediate fall in pressure and the respiration is much diminished.

mals a profound anesthesia may be readily obtained if all carbon dioxide effects be carefully avoided.

It seems probable that in average cases the heart and circulation are not much affected by the gas. Fig. 4 shows the result produced by injecting nitrous oxide into the breathing bag when the animal was already anesthetized by ethyl chloride. Three injections were made but the effects on both blood-pressure and respiration were practically nil. This corresponds very well to the injection of an ordinary drug solution into the femoral vein when an animal is anesthetized with ether. (Fig. 5 shows the reverse of this experiment and illustrates the action of ethyl chloride on an animal already anesthetized by nitrous oxide.) As a kind of check on these experiments another tracing (Fig. 6) is shown in which at two places a small amount of carbon dioxide was injected from a tank into the breathing bag. There is an immediate stimulation of the respiration and the blood pressure falls, probably from a direct action on the heart. The gas was

quickly emptied out and the bag was again refilled with nitrous oxide plus a suitable amount of oxygen. This shows quite well the action of even small amounts of carbon dioxide. I strongly suspect that some such action as this, either by excess of carbon dioxide, or from lack of oxygen, or both, constitutes the real cause of the undesirable after effects which are liable to follow from prolonged nitrous oxide anesthesia. And I am inclined to believe that these after effects may be very generally avoided by a correct and scientific administration of the nitrous oxide.

I have repeatedly observed, as have others, independently in my laboratory, that if one breathes a mixture of nitrous oxide and oxygen for a certain time, for example five minutes, and then passes under the influence of the gas to a given degree, he can then considerably increase the quantity of oxygen in the bag without lessening the influence of the nitrous oxide so far as the subject of

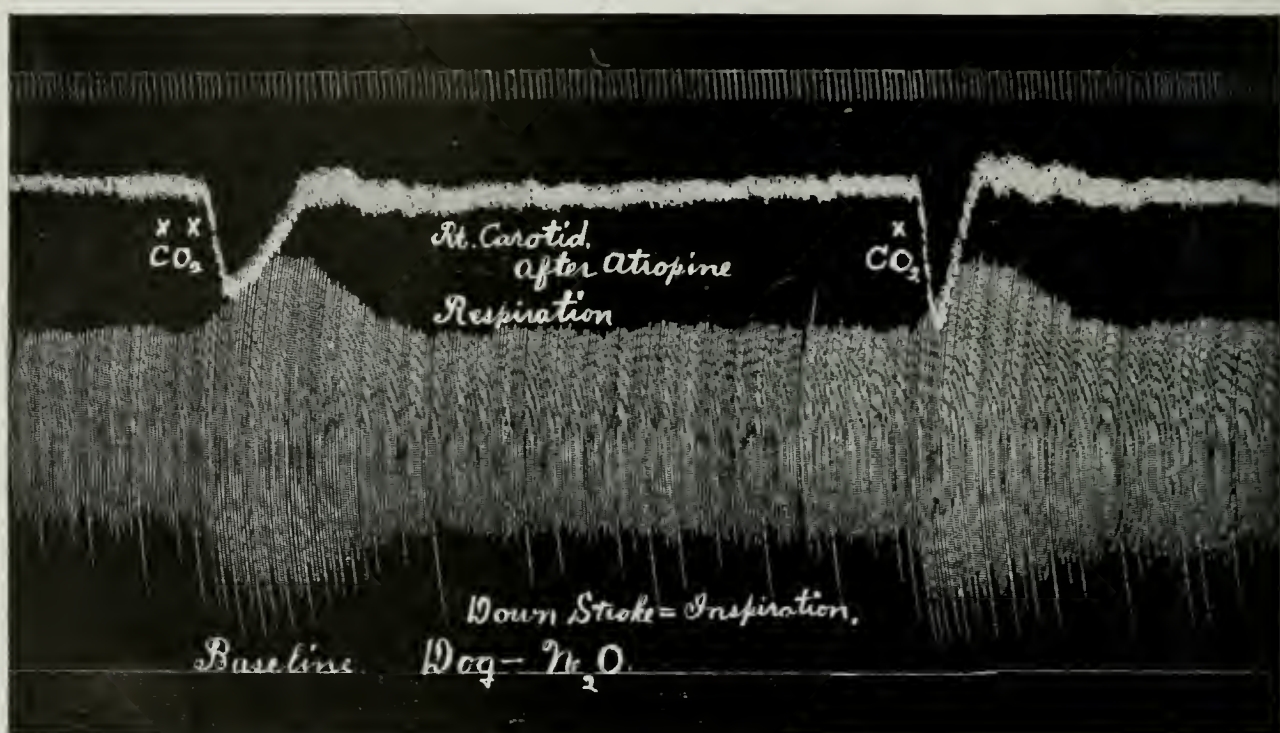


Fig. 6.—This tracing was made in a class experiment by Messrs. Mitchell, Day, Lueking and McKee. It shows the results produced on the respiration and blood pressure by injecting (twice) a small amount of carbon dioxide into the breathing bag while the dog was anesthetized by nitrous oxide. In each case the CO_2 was quickly emptied out of the bag after the animal began to show marked symptoms.

the experiment himself can determine. The reason for this appears to be as follows: On breathing the N_2O at first the whole body of the subject after a time becomes saturated with the gas at the given partial pressure. (As the first portion of gas is absorbed, one can see the bag shrink fairly rapidly with animals.) More N_2O must be run into the bag to replace that absorbed. But after the anesthesia or analgesia has reached a given degree, then if no more gas, but only oxygen, is given, the effects of the N_2O on the subject should remain fairly constant. It will be noted that the gas is excreted only into the bag from which in a given time approximately the same quantity of N_2O will pass back again into the blood. Supposing the bag was filled to the amount of two gallons* with 90 per cent N_2O and 10 per cent oxygen. If then one adds a quart of oxygen to the bag the per cent of oxygen the patient would breathe should be increased by

one-ninth of the total amount of mixed gases in the bag after the quart of oxygen is added. It would appear that this oxygen should be readily absorbed by the lungs in approximately the same proportion and quantity as oxygen is absorbed by the blood from the air (which contains oxygen in about the same proportion as the bag would now contain it, i.e., about 20 per cent). This would probably not be quite correct, for nitrous oxide has some power to displace oxygen from its solution in water (Sir Humphrey Davy⁹), and this probably holds good for the blood in the pulmonary capillaries also. On the other hand, when the quart of oxygen is run into the bag, the latter will be expanded by a volume equal to one quart and into this space the nitrous oxide already in the bag and also that

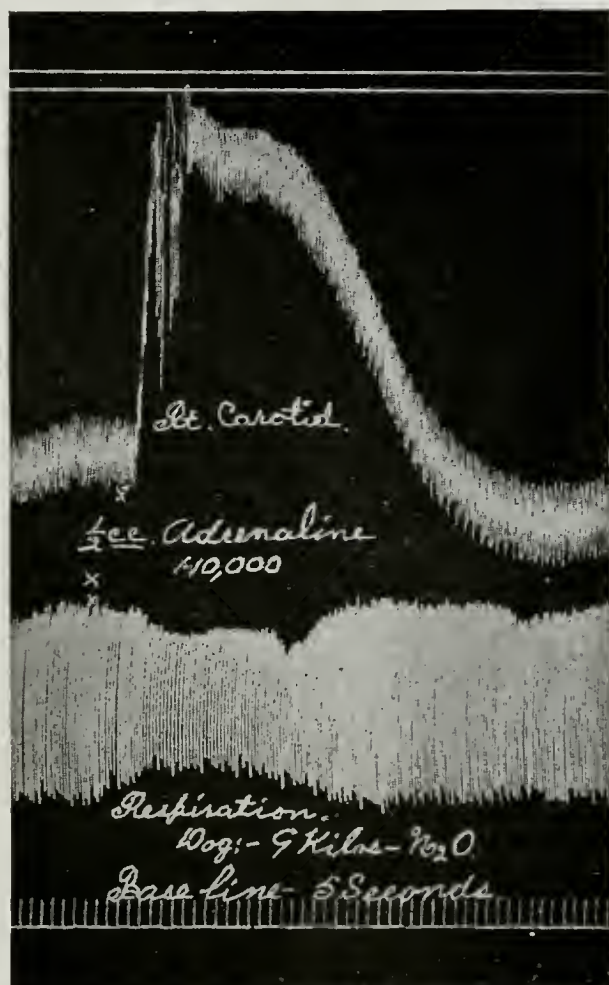


Fig. 7.—The animal was anesthetized with nitrous oxide. At the point indicated adrenaline ($\frac{1}{10}$ c.c., 1-10,000) was injected intravenously. The vagi were intact. (For discussion, see text.)

dissolved in the blood and tissues of the subject, may diffuse. But if, for example, the blood and tissues of the subject had absorbed two gallons of N_2O and the bag contained two and one-fourth gallons of (mixed) gases after the quart of oxygen was added, then there would be a chance for the N_2O to be diluted by approximately one-seventeenth of the total volume of gases or 5.8 per cent. At that time the subject might be breathing almost 20 per cent of oxygen and this is readily absorbed by the hemoglobin of the blood. In other words, the relative increase in percentage of oxygen breathed when a given amount of oxygen is added to the bag, is greater than is the relative amount of dilution of the nitrous oxide with which the subject is saturated after the oxygen is added to the bag.

It was shown by Van Arsdale¹⁰ in 1891 that the breathing of nitrous oxide to and fro from a bag in which the gas (plus the desired amount of oxygen) was contained at an increased pressure above that of the atmosphere caused an increase in the depth of the narcosis produced. (This was an entirely different principle from that which Paul Bert¹¹ and later Claude Martin¹² used in which the patient or animal was placed in an air-tight room, the air pressure in which was raised one-fourth above that of the atmosphere after which 80% N₂O plus 20% oxygen was administered to the patient or animal.) I have tried to verify Van Arsdale's results many times. In some cases (with dogs) increasing the pressure of the gas in the bag does deepen the anesthesia, but in many other cases I have not been able to demonstrate any advantage from this increased pressure.

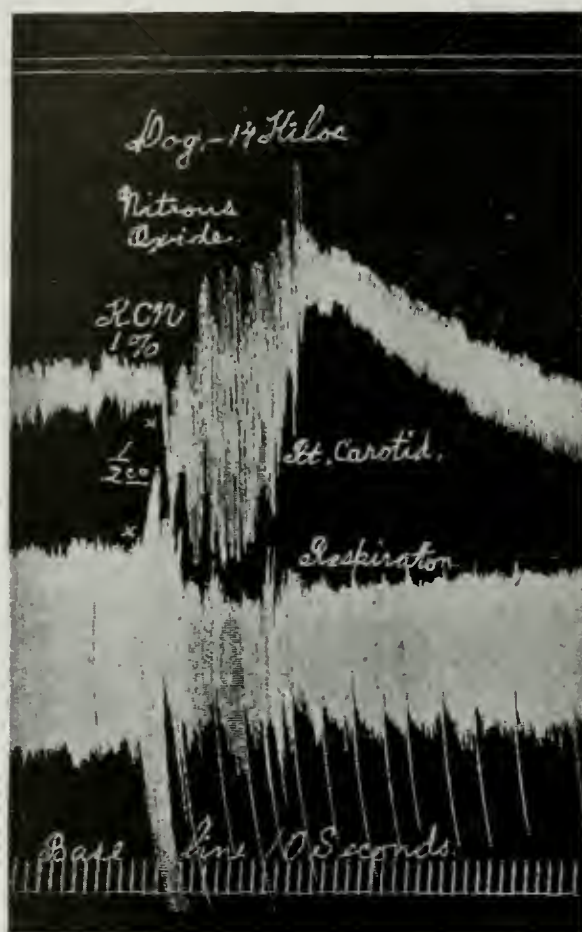


Fig. 8.—Dog anesthetized with nitrous oxide. At the point indicated $\frac{1}{2}$ c.c. of 1% KCN was injected intravenously. The vagi were intact. (For discussion, see text.)

Perhaps the increased respiratory exertion, the marked hindrance to the pulmonary circulation and the attendant obstruction of gaseous exchange in the lungs were sufficient in many cases to overcome the advantages of the increase in absorption of the N₂O which the raised pressure might bring about.

The intravenous injection of adrenaline in an animal under nitrous oxide anesthesia gives a practically normal reaction, i.e., the record is almost exactly like that produced by adrenaline in an animal under ether. The rise in pressure here probably supplies more oxygen to the brain and whatever asphyxia may have been present from the administration of the nitrous oxide is thereby reduced. (See Fig. 7.) On the other hand, the injection of cyanides (which are supposed to cause an internal asphyxia by lessening the tissue oxidations through

inhibiting ferment action) causes a markedly increased reaction both as regards the respiration and the circulation. The animal also shows a more marked convulsive reaction than it does under ether. (See Fig. 8.) I have controlled this by anesthetizing the animal first with N_2O and obtaining records of the blood pressure and respiration from the cyanides and then giving the animal ether, after which more records were obtained.

The motor areas are much more sensitive under nitrous oxide than under ether. One can easily secure very extensive movements of the muscles of the opposite side and can readily pick out the areas for individual groups of muscles. I have observed that dogs under nitrous oxide anesthesia may not well withstand extensive operations, particularly if the abdomen is opened and the viscera manipulated in any way.

In several respects there is a striking similarity between the effects of nitrous

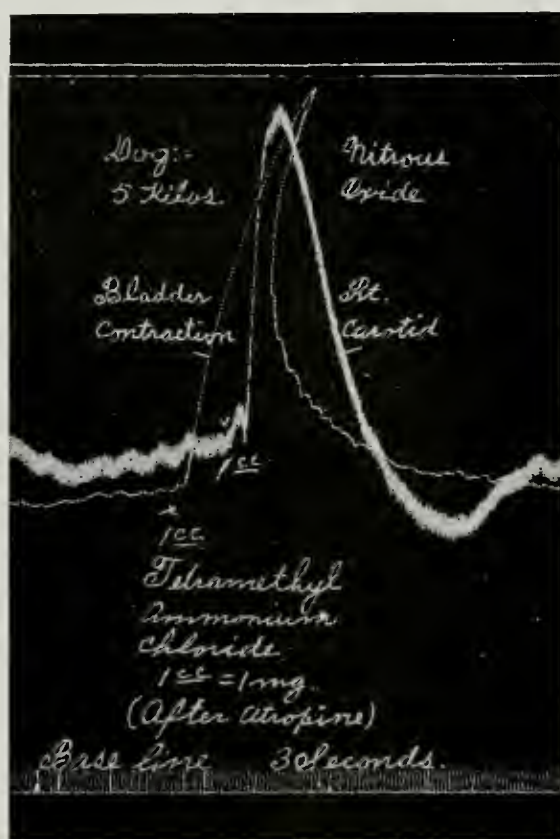


Fig. 9.—Dog anesthetized with nitrous oxide. The tracing shows the blood pressure (Rt. carotid) and the bladder contractions (up-stroke). At the point indicated 1 c.c. of tetramethylammoniumchloride was injected intravenously. The animal had previously received $1\frac{1}{2}$ mgs. of atropine.

oxide and those of morphine in dogs. Among these may be mentioned the production of Cheyne-Stokes respiration. This is generally present in prolonged anesthesia in dogs. The irritability of the cord is also much less depressed than is the case with the methane series of anesthetics and this action also closely resembles that of morphine. As under morphine defecation also sometimes occurs, but I have generally been inclined to attribute this to asphyxia, although other factors may be involved. A peculiar feature is often noticed in the fact that the dogs, while lying quietly and apparently fairly well anesthetized, may be aroused and waked up by stimulation or shaking in a manner very similar to

*For clearness of description I have assumed that the volume of one gallon of the gas may be considered equal to the volume of four quarts. We need not consider variations of temperature, pressure, etc.

that possible under a moderate dose of morphine. When thus aroused there is also often observed a marked acceleration and increase in strength of the heart beat. If the animal be again left alone it will soon return into the somnolent, or perhaps analgesic state, very much as occurs after morphine. It is difficult to study the analgesic effect of nitrous oxide separately and apart from the production of total unconsciousness in dogs, for these animals, so long as they are conscious, are very likely to struggle and try to escape even though they feel no pain whatsoever.

The thought has occurred to me many times that nitrous oxide might be used as a hypnotic. By ordinary methods of administration this is obviously impractical. But by a slight modification of the apparatus which I have used I am inclined to believe this idea might be very well put into practice. I have tried

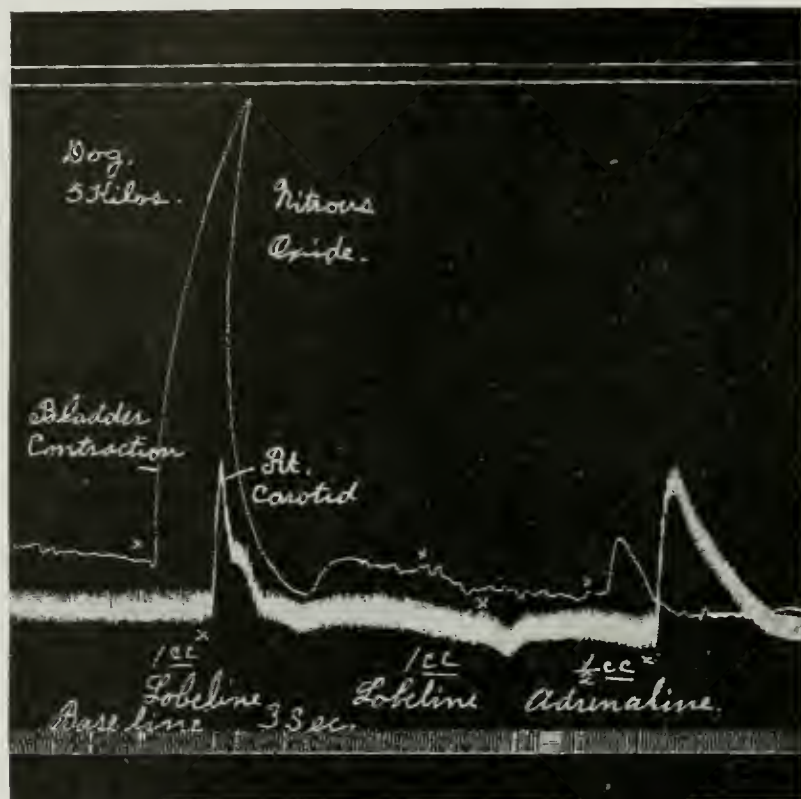


Fig. 10.—Dog under nitrous oxide anesthesia. Bladder contractions (up-stroke) and blood pressure. At the point indicated (on the left) 1 c.c. of lobeline was given intravenously. A marked contraction of the bladder and a small rise in blood pressure were produced. A little later a second dose of 1 c.c. of lobeline was given. Almost no results were produced by this, showing that the first dose of lobeline had produced ganglionic paralysis. Later a small dose ($\frac{1}{2}$ c.c.) of adrenaline was injected. This gave a slight bladder contraction and a small rise in blood pressure.

repeatedly to compare the mild on-coming effects of the gas as breathed with a considerable proportion of oxygen with the physical and mental sensations present as one begins to fall asleep. There is a very striking similarity, a marked feeling of tiredness and exhaustion, the limbs feel heavy and the eyelids tend to close. One's mentality gradually sinks and there is difficulty in maintaining connected thought. The natural inclination of the subject of the experiment is to lie down quietly and fall asleep. The sensations remind one of the feelings of a child worn out by a long day's play when it lies down at night to sleep. Sometimes I have noted slight muscular twitchings or feeble jumping or convulsive moments. These would probably not occur if the gas were administered very slowly with plenty of oxygen and a sufficiently long period of time were used

to bring on the action of the drug. Suggestion appears to play a noticeable part in this action, for if one keeps perfectly quiet and at rest and tries to go to sleep, then the somnolent action of the gas is especially liable to be well marked. It would appear that this matter of suggestion extends even to dogs. For an animal which is petted and induced to lie down quietly and at complete rest may very often take the gas readily and peacefully fall asleep.

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OBSERVATIONS ON CHOLESTERIN RETENTION AS A FACTOR IN CELL-PROLIFERATION*

BY GEORGINE LUDEN, M.D., ROCHESTER, MINN.

THE influence of heredity, the cancer age, the relatively large percentage of recurrence in malignant conditions, and lesions produced by trauma are recognized by the medical profession throughout the world as important contributory factors in malignant cell-proliferation. It is well known, for example, that in certain persons a slight injury will result in the formation of a rapidly growing tumor—a kick during a football game may be followed by sarcoma,¹ the irritation from the ragged edge of a tooth, by inoperable carcinoma. In these cases, however, a mere spark, as it were, causes a conflagration. The injury itself may be considered only a provocative factor, not the real cause, in the formation of the malignant growth; sparks do not create a conflagration unless they fall where inflammable material has accumulated.

That the fundamental factor in malignant diseases, whatever it may be, is widely distributed throughout the organism seems evident from the literature reporting conditions in which more than one tissue has become involved in the process of degeneration. Such cases, although not frequent, are by no means exceptional. Bartlett² concludes that "multiple primary malignant tumors occur in approximately 0.2 per cent of all cases of malignant tumors."

In this connection attention should be drawn to the fact that the term "multiple primary tumors" has been used in the literature repeatedly without distinction for the simultaneous occurrence of (a) different types of carcinoma, (b) carcinoma and sarcoma, and (c) tumors of totally diverse histologic character. The relative frequency of these three varieties of multiple types of malignant growths may be indicated as follows:

1. *Carcinoma Duplex*. (A term originally used by de Vries³): conditions in which two types of carcinoma, or carcinoma and epithelioma occur simultaneously;

2. *Carcinoma and Sarcoma*: conditions in which malignant degeneration is observed simultaneously in both epithelial and connective tissue;

3. *Multiple Type of Primary Malignant Tumors*: conditions in which tumors of a totally diverse histologic character are found simultaneously. These are comparable to those reported by Walter⁴ and Hanseemann,⁵ and are not to be confounded with the class of malignant neoplasms known as teratomas.

As far as can be ascertained, 119 cases of carcinoma duplex, 41 cases of carcinoma and sarcoma, and 15 cases of the multiple type of primary tumors have been observed in man and reported in literature, including the cases reported by Harbitz.⁶ In addition, I have studied 2 cases of carcinoma duplex, 3 cases of carcinoma and sarcoma and 2 cases of the true multiple type of neoplasms. One of the latter has been reported previously.

Seven cases of carcinoma and sarcoma in rodents have been reported by

*From the Mayo Clinic, Rochester, Minn.

Woglom,⁸ Haaland,⁹ Russell,¹⁰ Loeb¹¹ and Nicholson,¹² and 2 cases of the spontaneous multiple type of primary tumors in dogs have been described by Bartlett. It must be especially emphasized that although Bartlett suggests the possibility that the etiology of the types of tumors found in each of his two dogs was different, "one being indirectly due to a metabolic disturbance, the other, the mixed tumor, being in some way connected with an embryonal anlage," none of the primary multiple type of tumors in man herein reviewed belong to the class known as teratomas.

Two other facts demonstrating the ubiquity within the organism of the fundamental factor in malignant cell-proliferation are the occurrence of malignant degeneration in originally benign tumors, commented on by many authorities, and the "precancerous stage" described by Wilson,¹³ MacCarty¹⁴ and others.

As regards the identity of the causative factor, the branches of science that seem most particularly well fitted for the investigation are bacteriology, parasitology, and chemistry. Bacteriology thus far has been unable to furnish any material evidence of bacterial origin of malignancy. Parasitology has yielded many brilliant "finds," none of which has stood a five-year test. Physiologic chemistry, on the other hand, while it has offered no complete explanation of cancer, has already supplied a number of data indicating that marked metabolic changes occur in and go hand in hand with the progress of malignant disease. Lewis and Benedict¹⁵ have called attention to the increase of the sugar content of the blood in carcinoma. Davis¹⁶ describes a hema-uro-chrom test giving valuable information in cases of malignant disease, which he does not claim to be specific for cancer, but considers indicative rather of an increased hemolysis, cytolysis and proteolysis. The nitrogen content has been studied by many observers and found increased both in the urine output and in the tumor-tissue (Robin,¹⁷ Mueller, Gaertig, Klemperer, Embden, Knoop, Langenstein¹⁸). Capella¹⁹ has noted a sulphur reaction positive in the urine of patients with cancer. Pentagna²⁰ has called attention to the presence and importance of glycogen in malignant tumors "which increases in proportion to the degree of malignancy exhibited by the neoplasm."

It has been universally conceded that normal cell-proliferation or growth, is a proliferation of cells *within* normal bounds, and that a neoplasm represents a form of cell-proliferation *exceeding* normal bounds. The question then naturally arises: Is there any chemical substance which appears to be essential for normal proliferation, or growth, the activity of which may be traced in abnormal proliferation, or malignancy?

The following facts, I believe, seem to indicate that such a substance is cholesterin.

Dorée, Ellis and Gardner²¹ have shown by chemical analysis that the cholesterin content of the egg-yolk diminishes proportionally to the growth of the chick embryo, cholesterin being used in the process of cell-proliferation and assimilated in such a way that it cannot be recovered in the same quantity from the mass of cells representing the embryo. Aschoff²² and Autenrieth and Funk²³ have called attention to the increase of cholesterin in the blood during pregnancy. Bacmeister and Havers²⁴ demonstrated that, whereas the cholesterin content in the blood of pregnant bitches increases until the pups are littered, it gradually

returns to normal shortly afterward. Burnett and Robertson²⁵ in experimenting with tumor-grafted rats, showed that the intravenous injection of cholesterolin-sodium-oleate emulsion causes the tumors in the injected animals to double in size in a given time those in the tumor-bearing controls, all the animals having been grafted at the same time and with the same tumor. Browder²⁶ has proved that the addition of 0.01 gm. of cholesterolin to the culture medium increases the rate of division in paramecia from 1.33 to 5 times, as compared with controls grown in the same culture medium without added cholesterolin.

The above observations strongly suggest the existence of some kind of correlation between cholesterolin-increase and cell-proliferation, both under normal and abnormal conditions, and appear to indicate not only that cholesterolin is associated with active cell-proliferation, but also that it acts as a stimulant to cell division.

Before enumerating other facts based on experimental and clinical data which seem to support the above deduction concerning the physiologic activity



Fig. 1.—(85046.) Malignant growth involving upper third of ulna and radius—osteochondroma sarcoma. Tumor developed within three months at site of injury sustained two years previous. Showing proliferation of the bone. (H.E. $\times 120$ diam.)

of cholesterolin as related to cell-proliferation, a few words may be said in regard to the organs that regulate cholesterolin metabolism and its elimination. Named in the order of their apparent relative importance they are as follows:

1. Adrenal (Rothschild,²⁷ Sternberg,²⁸ Landau,²⁹ Gardner and Lander,³⁰ Stewart,³¹ Weltmann,³² Hueck,³³ McMeans³⁴).
2. Liver (Rothschild, Weltmann, Anitschkow and Chalatow,³⁵ McMeans).
3. Spleen and the "endothelial apparatus" (Aschoff and Landau,³⁶ Soper,³⁷ Rothschild, McMeans).
4. Ovary and corpus luteum (McMeans).

Apart from these organs of regulation, the body has at its command a number of other means by which, under normal conditions, it can maintain its cholesterolin balance. Surplus cholesterolin is eliminated, for example, in the feces (McNee³⁸) and the bile (Rothschild), and is stored in both the endothelial lining of the blood vessels, as in arteriosclerosis, (Klotz,³⁹ Saltykow,⁴⁰ Anitschkow,

Krylow,⁴¹ McMeans), and in the body-fat (Rothschild, Hueck, Wacker⁴²). In addition, there are what might be called "emergency safety valves," such as cholesterin cysts, boils, etc. An instance of the latter occurred in one of our cholesterin-fed goats which developed a boil, the contents of which had a cholesterin value of 0.70 per cent, about four times that of normal goats' blood.

Notwithstanding these natural means of defense against an accumulation

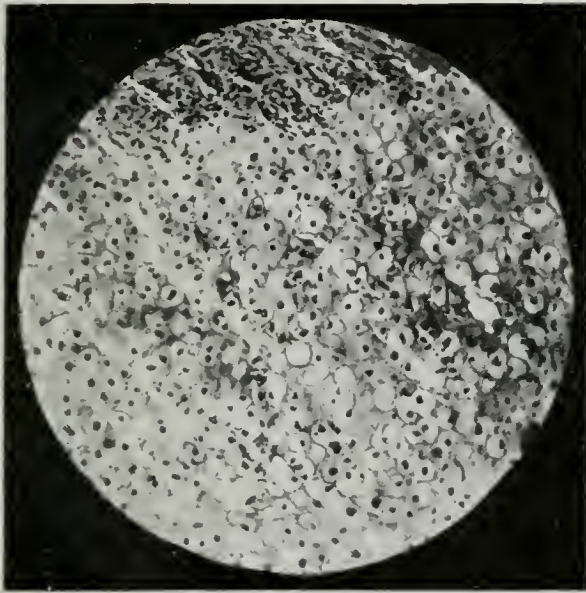


Fig. 2.—(Same as Fig. 1.) Proliferation of cartilage. (H.E. $\times 250$ diam.)

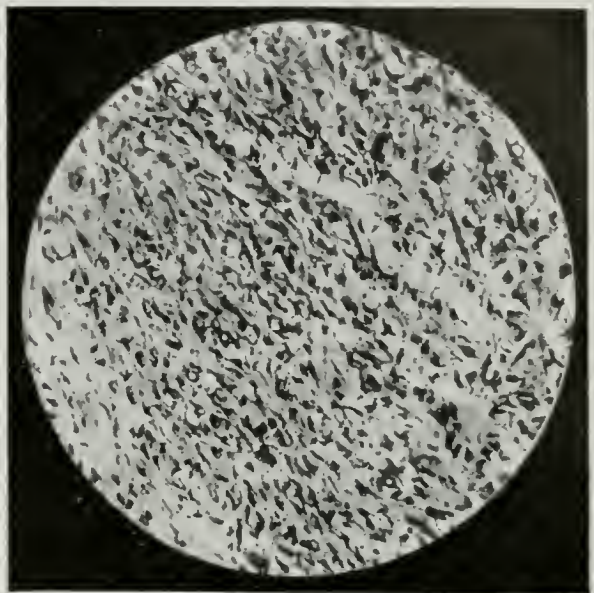


Fig. 3.—(Same as Fig. 1.) Proliferation of connective tissue. (H.E. $\times 250$ diam.)

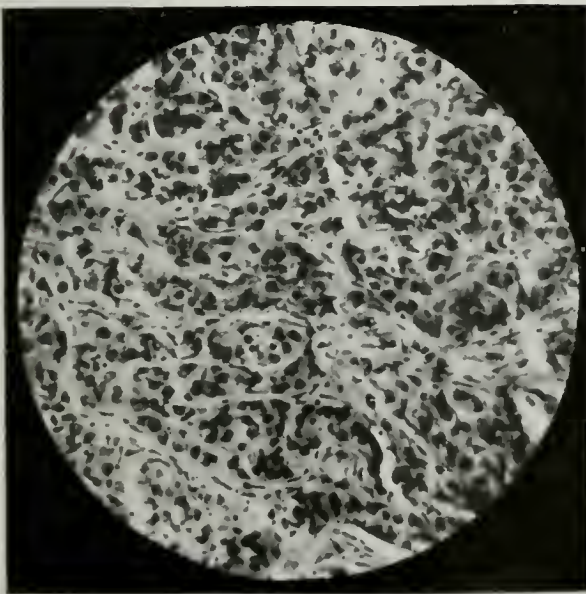


Fig. 4.—(Same as Fig. 1.) Proliferation of epithelium. (H.E. $\times 250$ diam.)

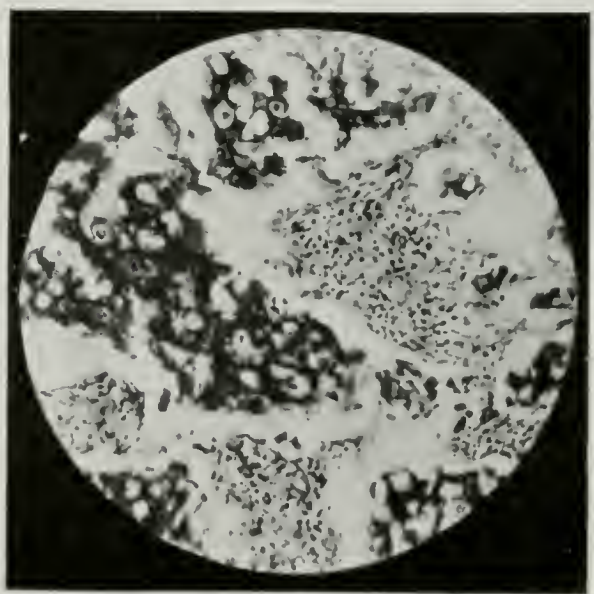


Fig. 5.—(Same as Fig. 1.) Proliferation of the epithelium showing a different type of carcinoma (myxomatous degeneration). (H.E. $\times 250$ diam.)

of cholesterin, there occur at times symptoms which it seems possible to explain only as being due to cholesterin retention and these symptoms go hand in hand with cell-proliferation (Figs. 6 and 7).

A patient (No. 63175), male, aged 57, came to the Mayo Clinic in 1912 on account of "bladder trouble." An operation (prostatectomy) adeno-fibromatous hypertrophy was discovered, but no trace of a malignancy was noted

microscopically. Four years later the man returned in a critical condition and died within three weeks. At autopsy extensive atheromatosis with ulcerating plaques was found in the aorta, together with carcinomatous nodules in the prostatic bed, malignant proliferation of the epithelium and the sebaceous glands in the suprapubic scar. 'The skin was not broken.' It will be remembered that artificial atheromatosis has been produced in animals by prolonged cholesterol feeding (Anitschkow, Saltykow, Stuckey, McMeans) and that the atheromatous plaques were microscopically identical with those found in man.

Proceeding on the working hypothesis that essential factors for the effectual progress of malignant proliferation are,⁴³ (1) the creation of a disturbance of metabolism which would lead to an accumulation of cholesterol, (2) an extraneous irritant, and (3) the breaking down of the "lymphoid-defence," the importance of which has been established by the work of Murphy and Morton,⁴⁴ I began in March, 1915, to give light treatment by roentgen rays to a double-adrenal-

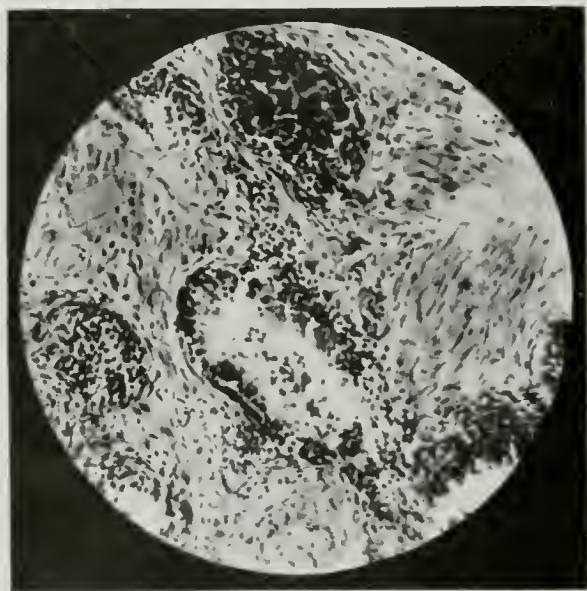


Fig. 6.—(63175.) Recurrent carcinoma of the prostate. (H.E. $\times 350$.)

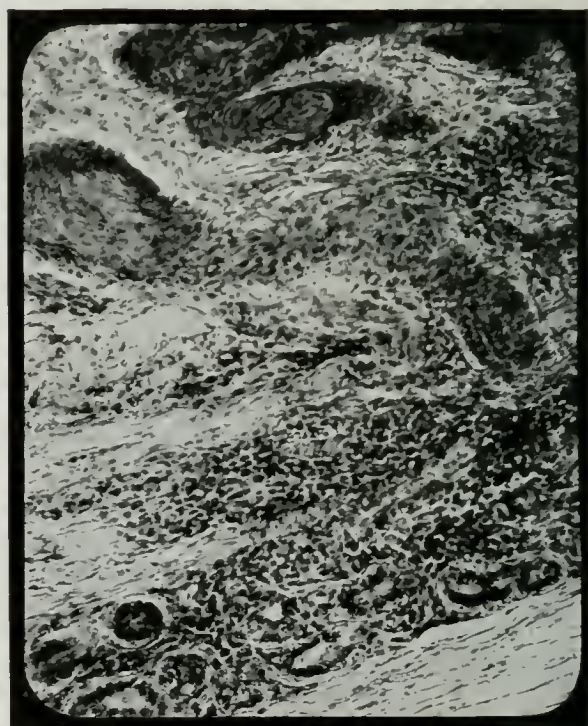


Fig. 7.—(Same as Fig. 6.) Malignant proliferation of the epithelium and sebaceous glands found at autopsy in operative sinus, four years after operation. (H.E. $\times 350$.)

ectomized spermophile (*Citellus tridecemlineatus*). Experiments conducted by Mann* had already shown that these spermophiles survived double adrenalectomy better than most animals. The results obtained were a marked change in the blood picture, there being an increase of normoblasts from 0 to 10 per cent in the course of six months, and the appearance of pathologic, "unripe" leukocytes together with proliferation in various tissues (Figs. 8 and 9). The experiment was then repeated on 8 spermophiles; 2 were double adrenalectomized, 3 were double-ovariectomized and 3 were used as normal controls. The results already described were found to repeat themselves both in blood and tissue.** The

*Observations to be published.

**Details will be reported in a future publication.

proliferation of the various types of tissues is comparable to the proliferation of the small arteries in lungs and kidneys together with changes in the spleen resembling those found in "Gaucher's spleen," which have been observed by McMeans after forced cholesterin feeding.

I have studied simultaneously in goats the effect of cholesterin feeding alone,



Fig. 8.—(Spermophile 28.) Proliferation of epithelial elements in the bladder of double adrenalectomized spermophile (*Citellus tridecemlineatus*), which had been given light roentgen treatment (42 Holzkecht in the course of 6 months at distance of 24 inches from the tube). (H.E. $\times 300$ diam.)



Fig. 9.—(Same as Fig. 8.) Proliferation in the outer zone of the kidney. (H.E. $\times 300$ diam.)

the influence of the roentgen rays alone, and the influence of cholesterin feeding combined with roentgen treatment. Here also similar changes in the blood picture were observed. Six goats were used for this experiment.

The blood of our six goats was examined weekly for a period of six weeks before the experiments were begun. During this time the animals were kept

under uniform and constant conditions, and no great variations in their blood picture occurred. The most marked changes were those produced by cholesterol feeding alone.** These changes in one of the goats (No. 21) are shown in Table I.

Recently I have made a series of observations on the cholesterol content of the blood of patients suffering from malignant disease. The Autenrieth-Funk⁴⁵ method and Bloor's⁴⁶ modification thereof were used. In some cases Bloor's test gave slightly higher values, in others the results were identical. The highest value has been given in the table. The normal cholesterol value as given by Authenrieth and Bloor averages about 0.18 with a low limit of 0.14 per cent. The high normal value has not yet been determined, but I have found it as high as 0.27 per cent in apparently normal adults. Authenrieth has found a cholesterol value as high as 0.30 per cent in the blood of normal pregnant women. In a case of xanthoma tuberosum he reports a cholesterol value in the blood of 0.58 per cent before operation and 0.54 per cent after operation.

TABLE I.—CHANGES IN THE BLOOD-PICTURE OF GOAT FED CHOLESTERIN ONLY (GOAT 21).
(Results expressed in per cent.)

Date 1915-1916.	Polymorphonuclear leukocytes.	Small lymphocytes.	Large lymphocytes.	Eosinophile leukocytes.	Mast-cells.	Transitionals,*	Neutrophile myelocytes	Normoblasts	Turk's "irritation cells."	Atypical small lymphocytes.	Tumor 1.**	Tumor 2.***	Cholesterol.	
Period I.—Control Period.														
Sept. 10	24	70.5	1.5	—	1.5	—	2	—	—	—	—	—	—	
Oct. 4	38	50	7.5	—	1	2	1.5	—	—	—	—	—	0.124	
18	30	60.5	7.5	1	1	—	—	—	—	—	—	—	0.084	
21	31	58.5	9.5	—	—	—	1	—	—	—	—	—	0.078	
25	38	53.5	5.5	—	1	1	1	—	—	—	—	—	0.07	
28	54.5	30.5	5.5	2	5	3	—	—	—	—	—	—	0.072	
Period 2.—0.30 Gm. Cholesterol Daily (Beginning Nov. 2).														
Nov. 8	28	25	25	10.5	4	6.5	.5	.5	—	—	—	—	0.15	
15	27.5	12.5	10	24	10.5	2	1.5	1	—	—	—	—	0.186	
22	26.5	14	10	15	7	2	4	3	.5	6	3	—	0.146	
Dec. 6	26.5	9	10	5	5	1	7	10	1.5	15	5	—	0.158	
13	44	2.5	6.5	.5	8	—	11	—	1.5	20	3.5	1.5	0.158	
20	33	8	24	2	1	4	11	4	2	12	9	2	0.14	
27	30.5	9	13.5	3	3.5	2	8	2	1	18	9	.5	0.186	
Jan. 5	25	8	19	2	7	6.5	10	2	—	18	2	1.5	0.168	
10	30	5	12.5	3	10	4	12.5	4	—	9	9	1	0.158	
17	19	5	11	7	11	16	14	—	.5	15	8	1	0.14	
24	27	30	11	5.5	4.5	11.5	2.5	2						
									1 Meg.	—	5.5	.5	—	0.14
Feb. 1	38	27	12	4	4.5	8.5	1	—	—	3.5	—	1	0.13	
7	30	26.5	16	3	15	3.5	1.5	1.5	—	2.5	—	.5	0.13	

Hemoglobin, 20 per cent; erythrocytes, 16 million; leukocytes, 15,200; index, 0.7.

*Transitionals—variety of "large mononuclears," with kidney-shaped nucleus; the typical mononuclears with single round or slightly oval nucleus were not found in this count.
**Tumor 1.—term borrowed from Schleip's Atlas der Blutkrankheiten Leukosarcomatosis, p. 128, Fig. 61, with which the cells appeared to be identical.
***Tumor 2.—term borrowed from Schleip's Atlas (Carcinose des Knochenmarks), p. 124, Fig. 59, h., with which cells appeared to be identical.

The values found in the blood in the cases of malignancy that I examined are given in Table II.

Weltmann⁴⁷ has studied the cholesterin percentages found in the blood under various conditions of disease, and finds them relatively high in arteriosclerosis, nephritis, non-ulcerating tumors, disease of the liver, and diabetes, but as he uses another method (a modification of the Neumann-Hermann test with different scale and different color-reaction) and as he himself points out, "obtained not accurate but only comparative values," his data cannot be used for comparison with mine which are comparable only to Authenrieth's findings in the case of xanthoma tuberosum (0.58) referred to above.

As may be seen in Case 147020 (Table II), the patient having the highest cholesterin value of all showed not only symptoms of proliferation in the blood (109 normoblasts, 14 megaloblasts in a count of 300 cells; a picture typical of pernicious anemia according to Ehrlich; atypical according to Grawitz) but malignant degeneration in a number of histologically diverse tissues as well (Figs. 10 to 14).

In Case 140350 the formation of a rapidly growing, highly malignant tumor was preceded by a clinical history of unrecognized chronic appendicitis. The chronic inflammatory condition had lasted for several years and during this period the patient had gained considerably in weight. At operation uterine fibroids were found. After hysterectomy and unilateral ovariectomy, the men-

TABLE II.—CHOLESTERIN VALUES IN THE BLOOD OF PATIENTS WITH MALIGNANCY.

Number.	Sex.	Age Years.	Civil State.	Diagnosis.	Treatment.	Cholesterin Per cent.*
1- 147020	F	35	M	General carcinomatosis with blood picture of pernicious anemia	Transfusions Autopsy	0.710
2- 154152	F	55	M	Epithelioma of tongue	Before treatment**	0.502
3- 136512	F	39	M	Pernicious anemia (?)	Two trans- fusions	0.502
4- 152680	F	52	M	Epithelioma of tongue	Before treatment	0.474
5- 150864	F	49	M	Mass in the sigmoid (benign?)+	Before treatment	0.446
6- 137213	M	45	M	Recurrent cancer of lower jaw	Before treatment	0.446
7- 150350	F	48	M	Epithelioma of the arm; amputation++ (exarticulation at shoulder)	am-radium; treatments	0.446
8- 151635	M	52	M	Cancer (?) of the tongue, blastomycosis	Potassium iodide; iodine locally; radium	0.266
9- 133093	F	65	M	Multiple lymphomas	Operation; radium; Fowler's solution	0.254

*Average of 10 to 12 readings on each specimen of blood examined.

**Radium appears to lower cholesterin values.

+Blood for cholesterin test was taken during anesthesia for operation; patient had been under the anesthetic for 15 minutes. The effect of ether-anesthesia on cholesterin values is being studied; the values seem to be lowered by anesthesia and by radium treatment.

++The sigmoid and colon descendens appeared to be involved though the character of the tumor could not be defined. Since the patient was in fairly poor health and did not present any clinical symptoms of malignancy, operation which would probably have meant the resection of two-thirds of the large intestine seemed contraindicated. The clinicians expressed the opinion, however, that operation might be necessary eventually on account of obstruction or the development of malignant symptoms.

strual period disappeared completely. A year later slight injury to a birthmark, which had never given any trouble (patient's age 47) resulted within two months in the development of a highly malignant tumor (Figs. 18 to 20). Five points in the history of this patient seem to deserve special attention : (1) a condition of chronic infection, which, existing during a considerable period, is likely to have overtaxed the adrenal, the chief organ of regulation in cholesterol metabolism,



Fig. 10.—(147420.) Multiple primary tumors, atypical form of pernicious anemia, high cholesterol value in the blood (0.71%), malignant proliferation of the gastric mucosa. (H.E. $\times 250$ diam.)

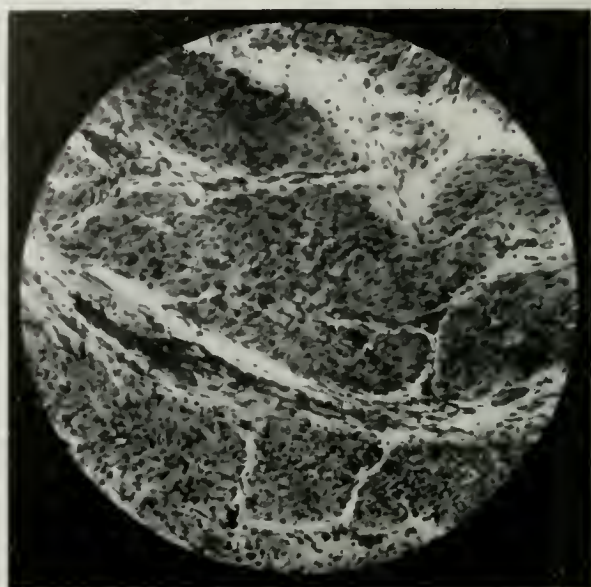


Fig. 12.—(Same as Fig. 10.) Invasion of the outer muscular strata. (H.E. $\times 600$ diam.)

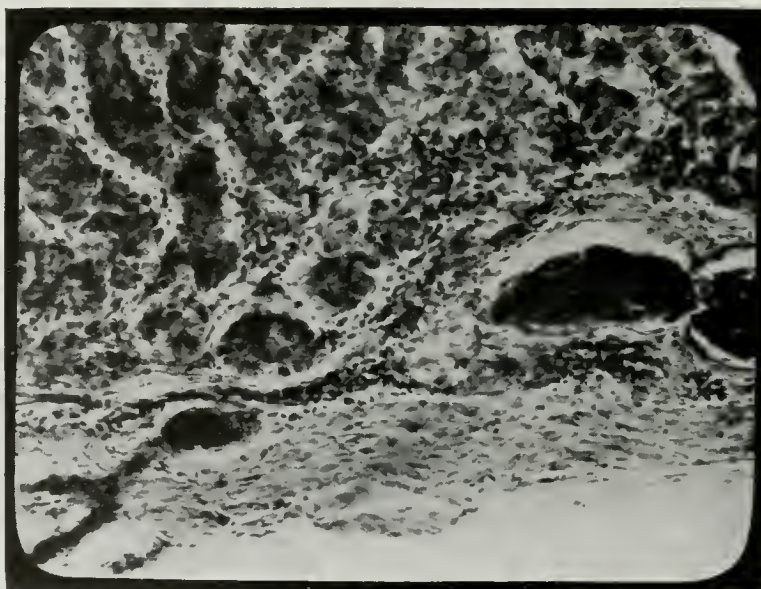


Fig. 11.—(Same as Fig. 10.) Malignant invasion of the muscularis mucosa. (H.E. $\times 350$ diam.)

(2) the increase of body-fat observed in the patient, which must be considered a measure of defense against the accumulation of cholesterol, as has been stated by Rothschild, (3) the high cholesterol value of the blood, (4) the benign tumors found at operation which may be considered another evidence of existing hypercholesteremia, and (5) the highly malignant tumor which developed within a year after cessation of the menses and which may be said to suggest that reproductive activity and malignant growth are more closely related than has been universally believed.

The influence of the sex glands on cell proliferation has been studied by a number of writers (Graf, Almagie, Rohdenburg, Bullock, Johnson, Hilario⁴⁸), the results of these investigations in many cases leading to conclusions diametrically opposed. Sweet, Corson-White and Saxon⁴⁹ found that "the receptivity for a tumor with moderate powers of proliferation is increased by castration and that the proliferative power of the neoplasm is augmented." The effect of

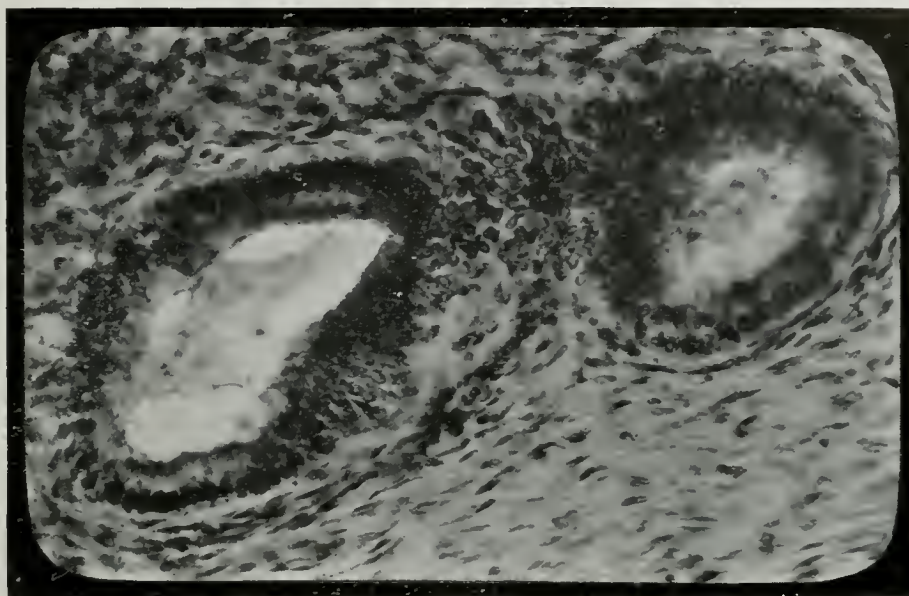


Fig. 13.—(Same as Fig. 10.) Malignant proliferation in the uterine glands (compare with Fig. 6). (H.E. $\times 600$ diam.)

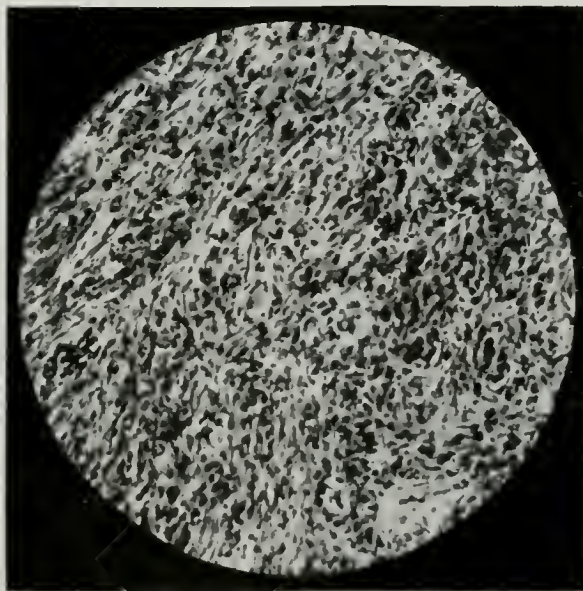


Fig. 14.—(Same as Fig. 10.) Left ovary, tumor 15 cm. diameter. Diffuse type of carcinoma. (H.E. $\times 350$ diam.)

castration on the formation of benign tumors in the antlers of deer has been commented on by Lauterborn⁵⁰ and Tandler and Gross.⁵¹

Recently Lathrop and Loeb⁵² have called attention to the influence of castration in young mice under six months by which the cancer incidence is decreased and the cancer age increased. They mention also "that non-breeding mice become fat at the age of 9 months, and at 15 months almost all are extremely fat—a change usually associated with a decline in the breeding activity." The fact that in these experiments, castration exercised a restraining influence on the

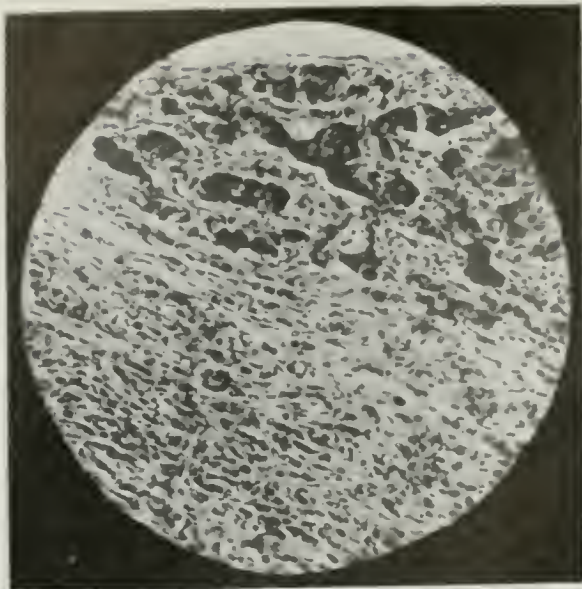


Fig. 15.—(Same as Fig. 10.) Tumor of right ovary, 6 cm. in diameter. Two types of carcinoma. Diffuse type mixed with solid strands. (H.E. \times 350 diam.)

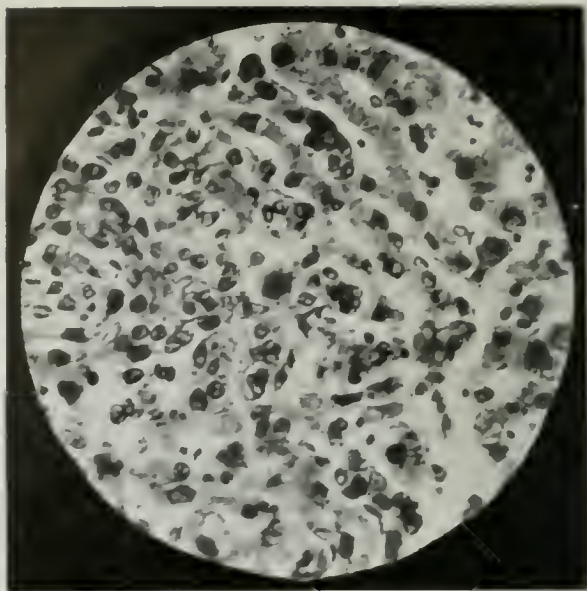


Fig. 16.—(Same as Fig. 10.) Malignant degeneration of the liver "trabecula." This type of malignancy was observed in every section. No metastatic nodules were found. (H.E. \times 300 diam.)

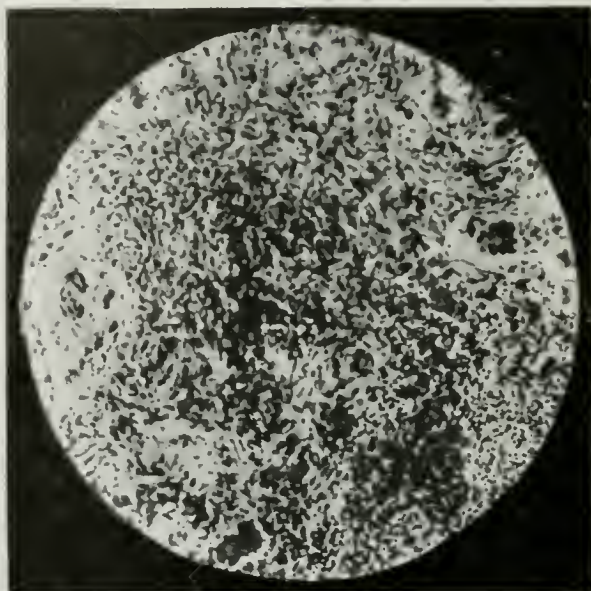


Fig. 17-A.

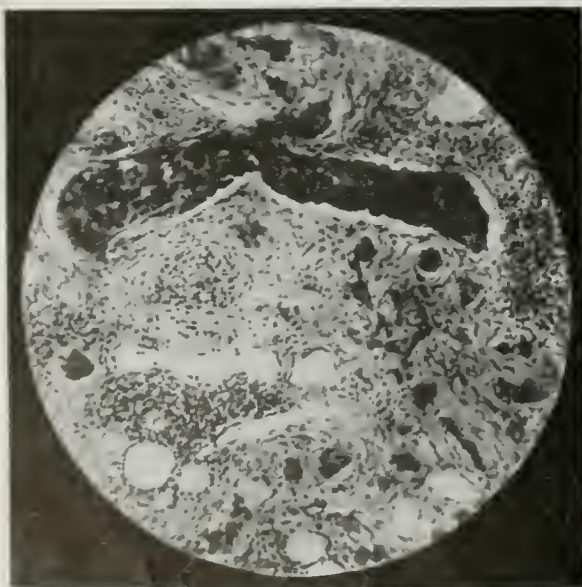


Fig. 17-B.

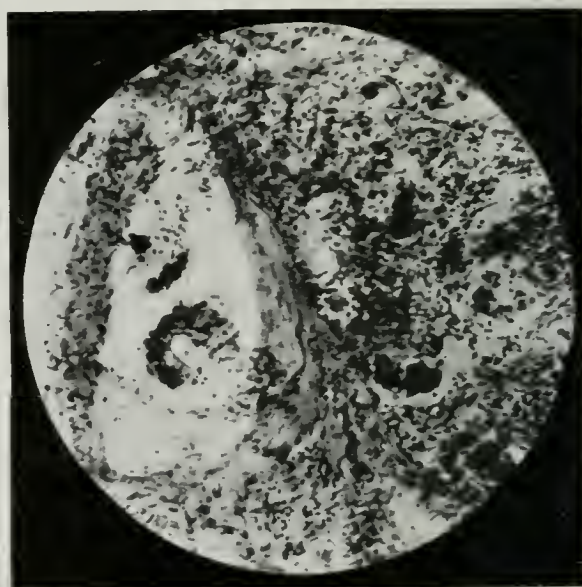


Fig. 17-C.

Fig. 17. A, B, C.—(Same as Fig. 10.) Lumbar lymphatic gland containing three different types of carcinoma of metastatic origin (A, diffuse; B, solid strand; C, adenoma). (H.E. \times 250 diam.)

progress of malignancy only when the operation was performed on young animals seems to suggest that the young organism, not yet entirely grown, might be able to utilize any surplus of cholesterin resulting from castration for purposes of natural growth, or that it has greater powers of elimination at its command. I was able to observe a distinct increase of cholesterin in the blood of goats following castration and the same observation has been made by Lowenthal⁵³ in rabbits. The increase of body fat usually observed in castrates and noted by

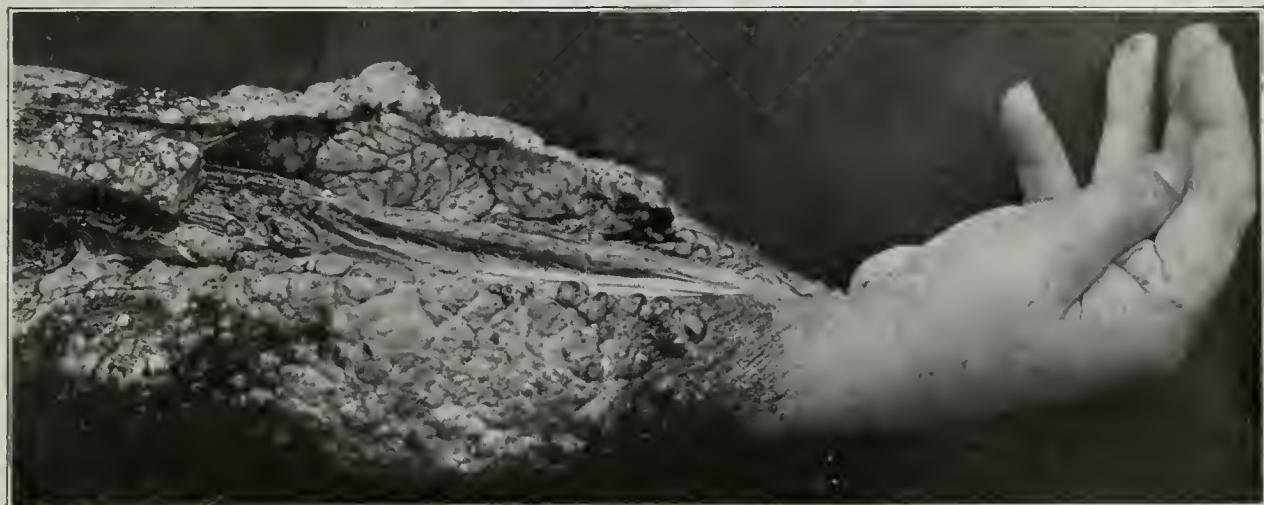


Fig. 18.—(150350.) Melano-epithelioma originating from a birthmark after slight injury.

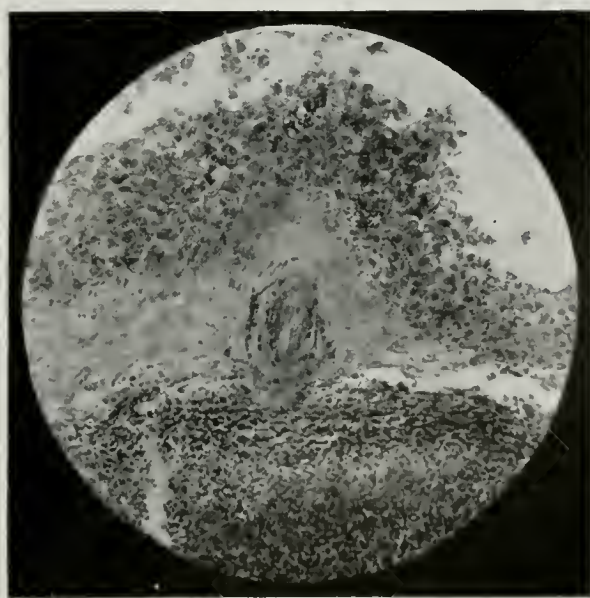


Fig. 19.—(Same as Fig. 18.) Carcinomatous portion of the tumor. (H.E. $\times 350$ diam.)

Lathrop and Loeb in their non-breeding mice would seem to indicate (in connection with other evidence concerning the physiologic activity of cholesterin) that the generative organs utilize a certain proportion of the available cholesterin for the formation of the reproductive cells.

SUMMARY.

1. Clinical data and experimental observation alike suggest that the causative factor in the production of malignant proliferation is widely distributed within the organism.

2. There is reason to believe that this fundamental factor is chemical in its nature.

3. The existence of a gradual transition between normal, reparative, benign and malignant proliferation has been commented on by many observers.

4. The evident connection observed between an increase of cholesterol and proliferation, both normal and abnormal, seems to suggest that cholesterol may act as a stimulant to cell-division.

5. After puberty and under normal conditions the process of cell-division is constantly demonstrable in the sex glands.

6. Recent investigations have shown that the sex glands appear to take an active (perhaps a prominent) part in the regulation of cholesterol metabolism.

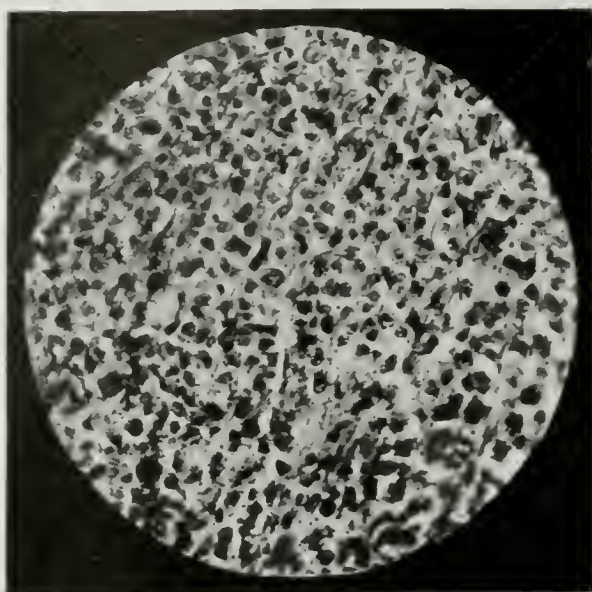


Fig. 20.—(Same as Fig. 18.) Sarcomatous portion of the tumor. (H.E. $\times 800$ diam.)

As cholesterol is stored in considerable quantities in the corpus luteum during pregnancy, and increases in the blood after castration, it seems permissible to assume that it may in some way stimulate the formation of the generative cells.

CONCLUSION.

These observations, the fact that the cancer age coincides with the cessation of reproductive activity as shown by the cancer statistics (Prinzing⁵⁴), the importance of metabolism emphasized by Hoffmann in his statistical review on the cancer rates of the Western Hemisphere, and the influence of heredity (the transmission of inadequate organs) established by the work of Slye,⁵⁵ all seem to support the view that the retention of cholesterol due to its insufficient conversion or defective elimination may be a primary factor in the etiology of malignant disease.

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THE PRESENT STATUS OF TUBERCULOSIS CHEMOTHERAPY *

BY LYDIA M. DEWITT, CHICAGO, ILL.

TUBERCULOSIS is a disease of so irregular a course, with such a tendency to periods of latency or intermission, alternating with periods of acute exacerbation and long periods of slow, chronic progress, that it is most deceptive in its therapy. Any treatment begun just before an intermission seems to be the cause of the apparent improvement, while one begun before an exacerbation is regarded as injurious. These facts make a judgment based on clinical evidence unreliable unless it extends over long periods and includes a large number of patients. Among experimental animals, moreover, we have some, like the guinea-pig, so extremely susceptible that they seem to have no natural forces of resistance and the disease is almost uniformly fatal regardless of treatment. If anything would cure the disease in guinea-pigs, therefore, it would almost certainly do so in man, who is much less susceptible. But it is quite possible that some treatment might cure the disease in man which would have little or no effect in the guinea-pig. The rabbit and the mouse, on the other hand, are much more resistant to human tuberculosis than is the guinea-pig, but the pathologic character of the disease produced by infection is quite unlike human tuberculosis. Hence no experimental animal has yet been found in which conditions are quite comparable to those in man. It is necessary, therefore, to use the available experimental animals in testing toxicity, determining dosage and palliative, if not curative effect; in other words in "getting a lead" as to the usefulness of any proposed method of treatment. If we find that, in a large series of animals as susceptible as the guinea-pig, a given treatment uniformly and consistently delays death, lessens the dissemination of the tubercles, increases connective tissue formation and diminishes the virulence and virility of the tubercle bacillus, even though the infection is not cured, we have a right to conclude that it is worthy of consideration and of trial on human patients. Even then it must be subjected to long periods of trial on large numbers of patients before it can be accepted as of value in tuberculosis therapy.

Since the failure of tuberculin in all its forms as a specific cure for the disease, investigators have turned almost with one accord to chemotherapy, believing that in the vast number of chemical substances and the endless variety of possible combinations of these, some might be found having a specific beneficial action on tuberculosis. These investigators have been encouraged by the success of Ehrlich and his coworkers in protozoan and spirochetal infections. In tuberculosis, however, we have quite a different problem. Instead of a delicate, naked organism circulating in the blood stream, we have an organism protected by a difficultly permeable membrane and rarely found in the circulating blood, but usually in nonvascular and often necrotic cells or groups of cells. These necrotic masses are readily permeated by crystalloid and semicrystalloid substances, which influence the tubercle favorably or unfavorably, first by break-

*From the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the University of Chicago.

ing down the tissue and permitting its absorption, second by favoring connective-tissue formation and calcification (Nature's method of effecting a cure); third, by attacking the bacilli themselves, whether intra- or extracellular. Certain true colloids also may be taken up by phagocytic cells and carried to the tubercles. The principal methods of attack are: (a) direct, on the organism of the disease, killing it or inhibiting its growth; and (b) indirect, on the protective agencies of the body.

Relatively few chemical compounds have as yet been tested with relation to their value in tuberculosis and it is the purpose of this paper to give briefly the present status of chemotherapy in tuberculosis.

IODINE.

Iodine is one of the substances longest used in the treatment of tuberculous patients. Its earlier use was no doubt purely empirical. In 1905, however, Cantacuzène,¹ as the result of an experimental study, reported that fat-free tubercle bacilli lose much of their toxicity if treated with an iodine solution previous to their injection and that they are then absorbed much more readily than the noniodized bacilli. He also states that fat-free bacilli and tubercles formed from them in guinea-pigs are much more rapidly absorbed if potassium iodide is administered to the animals daily, the iodine salt stimulating the phagocytic power of the endothelial cells. Hirsch² repeated these experiments and enlarged upon them, but was unable to verify the results obtained by Cantacuzène. Hirsch concludes that there is no experimental proof that iodine and iodides facilitate the absorption of necrotic tissue and organization of tuberculous and other granulation tissues. The daily administration of potassium iodide does not hasten the removal of tubercle bacilli by stimulating the phagocytic power of endothelial cells. Iodized fat-free tubercle bacilli are absorbed no faster than are the noniodized. Loeb and Michaud³ found that, if iodine in various forms was injected into tuberculous animals, much more iodine was found in tuberculous than in normal tissues. This finding was confirmed by Wells and Hedenburg,⁴ who, however, found that this property was not specific for tuberculous tissues, but was common to other necrotic tissues and due to the fact that dead and injured cells are more easily permeated by crystalloids. Lewis⁵ also found a greater amount of iodine in tuberculous than in normal tissues of animals treated with iodine compounds, but he also found more iodine in tuberculous than in normal tissues of animals not intentionally treated with iodine. No systematic experimental work has as yet been published on the therapeutic action of iodine in tuberculosis. DeWitt and Sherman⁶ found that Lugol's solution, full strength, could not be depended upon to kill tubercle bacilli in twenty-four hours and I have as yet found little, if any, therapeutic effect from the use of various iodine dye compounds used by me in the treatment of tuberculous guinea-pigs, although von Linden claims considerable value for her iodine compound of methylene blue.

ARSENIC.

Arsenic also is one of the drugs long used more or less empirically in the treatment of tuberculosis and it is natural that, after the success attending its use in certain spirochete infections, the attention of investigators on the chemo-

therapy of tuberculosis should be turned to arsenic and its compounds. In 1914, Schumacher⁷ published a communication on salvarsan as a true dye, in which he points out that arsenic destroys many bacteria and other disease agents to which it is able to gain access, but ascribes its slight effect on the tubercle and related organisms to the fact that their waxy envelope prevents penetration and thus protects them from the toxic influence of the arsenic. He suggests that salvarsan might be made more effective by combining it with some substance which could injure the tubercle bacillus in the body, such as cholin. He also suggests combining it with gold or gold-containing dye, so constituted that colloidal gold could be split off. Charpentier⁸ finds that tubercle bacilli grow well in the presence of sodium arsenate or of atoxyl; not quite so well in methyl-arsenate of sodium or cacodylate of sodium. Chemical tests show arsenic in the bacillary masses grown on media containing arsenic; he also finds arsenic in media to which he has added no arsenic, a fact which he explains by the presence of arsenic in most animal tissues and hence in protein containing media. He also finds that sodium cacodylate in no way modifies the progress of a tuberculous infection in guinea-pigs inoculated with virulent cultures and ascribes its favorable influence in human tuberculosis to its power to increase the natural forces of resistance which are present in man but not in the guinea-pig. In 1914, in a communication by Wells, DeWitt and Corper⁹ a reference was made to work being carried on by Corper and Arkin on the action of arsenic in experimental tuberculosis. The complete report of their work has just appeared in the *Journal of Infectious Diseases*.³³ They used sodium arsenate, sodium cacodylate, atoxyl, arsacetin and neosalvarsan. These preparations do not kill the tubercle bacillus *in vitro*; they penetrate tuberculous tissues, but not specifically; they have no effect on the progress of the disease in animals.

SODIUM SULPHOCYANATE.

Sodium sulphocyanate was also tested by Corper¹⁰ and found to have no chemical affinity for tuberculous tissues and no bactericidal effect on tubercle bacilli *in vitro*.

DYES—VITAL STAINS.

Dyes which can be introduced into the living body without material injury have for several years interested therapists in several fields. Thus Ehrlich began his famous series of experiments on the chemotherapy of spirochete infections with the dye trypan red, which was itself trypanocidal *in vivo*. Wassermann used eosin as a carrier for selenium in his cancer experiments. Ehrlich found methylene blue of some value in the treatment of neuralgia and malaria, and Einhorn used it as early as 1891 in the treatment of pulmonary tuberculosis. Lewis is experimenting with compounds of trypan red and trypan blue in the treatment of tuberculosis, and Weil¹¹ has recently published a communication on the use of certain dyes and dye compounds in the study and treatment of rat tumors. Congo red and some of its compounds were used without therapeutic effect, though they penetrated the tumors.

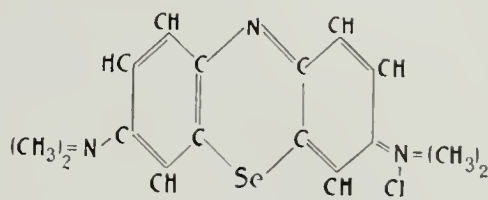
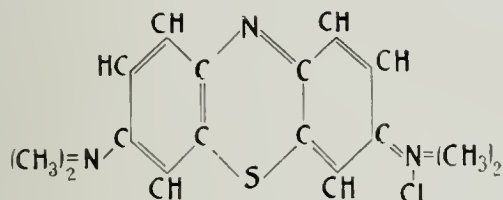
With my assistants and coworkers, I¹² have for some years been investigating numerous dyes and dye compounds with relation to experimental tuberculosis. In this study, we have endeavored to ascertain the power of the va-

rious dyes to penetrate and stain the tubercle and the individual tubercle bacilli; their ability to kill the tubercle bacillus or inhibit its growth; their effect on the development of experimental tuberculosis in animals. Because both tubercle bacilli and tubercles are rich in fats, it seemed reasonable to think that fat soluble dyes might be more efficient than fat insoluble ones. Sherman,¹⁴ however, found that the ordinary fat dyes, sudan iii, sudan yellow and sudan brown, scarlet R, Nile blue sulphate, etc., penetrate and stain the individual tubercle bacilli but little, if at all, although clumps and masses of culture show a mass stain. Corper¹⁵ also reported that these dyes, when administered to tuberculous guinea-pigs until the normal body fats were brilliantly stained, were never found within the tubercle and never stained the bacilli within the tubercles. The course of the tuberculous infection was never influenced favorably by the administration of these dyes, whether given in water or oil solutions, by injection or by mouth. As to dyes not soluble in fats, it was found by Goldmann¹⁵ and also by Lewis¹⁶ that the trypan dyes penetrated to the center of tubercles. I also ascertained that trypan blue easily penetrated and was retained in tubercles, whether young and epitheloid in character or having necrotic and caseous centers, and also into the large necrotic areas so common in the liver and spleen of tuberculous guinea-pigs. These dyes are found in certain phagocytic endothelial cells in the form of irregular, deeply stained, difficultly soluble granules, while in the caseous centers the dye is partly homogeneous and partly finely granular. The tubercle bacillus is stained but faintly, if at all, and is neither killed by the dyes nor inhibited in its growth. Not even is its virulence diminished, as animals infected with cultures which had been exposed to these dyes and inoculated animals treated with these dyes, develop the disease as rapidly and die as quickly of a severe generalized infection as do the untreated control animals. Lewis¹⁷ has also used trypan red compounded with iodine, thymus, eucalyptol, guaiacol and iodoform, with no appreciable influence in prolonging the course of the infection, although these compounds resemble the original dye in their power to penetrate the tubercle. DeWitt, also, in the hope of increasing its therapeutic power, compounded trypan blue with copper, silver, iron, and mercury. These salts were, however, more toxic and had little more influence on the course of the disease than the trypan blue, if we except the mercury compounds. The animals treated with these latter lived longer than the others and had very slight tuberculous involvement, either local or general.

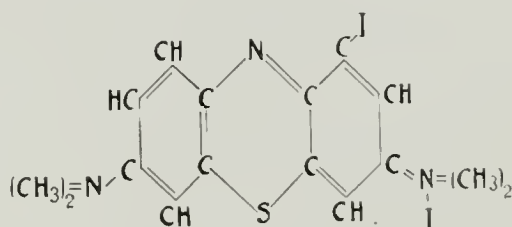
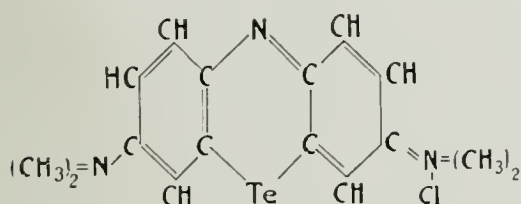
I also found that all the various methylene blues tested penetrated the tubercle and stained the tubercle bacilli *in vitro* very well; that basic fuchsin, crystal violet, erythrosyn and the eosins also stain the tubercle bacillus well; and that methylene blue, bismarck brown and brilliant cresyl blue have some bactericidal power and a marked inhibitory influence on the growth of the tubercle bacillus *in vitro*. The purified methylene blue of the pharmacopeia has but little general toxicity, though subcutaneous injections cause infiltration and ulcerations. Though methylene blue possesses so many qualities which should theoretically make it valuable as a therapeutic agent in tuberculosis, my experiments have not yet shown any considerable curative effect in the experimental disease in guinea-pigs. Von Linden reported rather better results than mine.

especially with the use of an iodine compound, the method of making which and the formula for which she does not divulge. She states that this dye prolonged life materially, caused healing of the initial sinus and abscess, increase of weight, lowering of fever and in one case perfect healing and sterilization.

Some modifications of methylene blue have been made for me. In one case, the sulphur of the methylene blue molecule was replaced by selenium and



in another by tellurium. These modifications were suggested to me by the finding of Gosio¹⁸ and Belfanti¹⁹ that the salts of selenium and tellurium have a pronounced bacteriotropic power over the tubercle bacillus and could perhaps be used as the point of origin for new therapeutic preparations. My selenium and tellurium blues were, however, weaker, less stable, and more toxic dyes



than methylene blue and showed no advantages over it. An iod-methylene blue has also been made for me, in which one iodine atom is in the ring. My experiments with this are still under way and will be reported in detail later.

HEAVY METALS.

In 1914, DeWitt and Sherman²⁰ published the results of a series of investigations on the bactericidal action of numerous chemical disinfectants on the human tubercle bacillus. Growth tests were made on artificial media and life and virulence tests on guinea-pigs. It was ascertained that 0.001 per cent of mercuric chloride killed within twenty-four hours all organisms, if spread out in a thin layer on glass beads. It required 0.004 per cent to destroy their power of growth in twenty-four hours, if the organisms were clumped. Gold chloride came next in bactericidal power, 0.005 per cent killing within twenty-four hours all tubercle bacilli, if spread out in a thin layer on glass beads. If in clumps, it required 0.01 per cent to destroy their power of growth. It was stated by Koch²¹ that one part of gold cyanide in 2,000,000 parts of media completely inhibited the growth of tubercle bacilli, a statement which I have been able to verify. Silver nitrate is slightly less bactericidal for these organisms, 0.01 per cent killing them within twenty-four hours, if in thin layer and 0.05 per cent, if in clumps.

Copper chloride stands lowest of all the metals tested, as it required 5 per cent to kill in twenty-four hours all the bacilli, if spread out in a thin layer and even 25 per cent did not kill all if they were in clumps. Von Linden claimed

that one part of copper chloride in 1,000,000 parts of media inhibited the growth of tubercle bacilli and that one part in 100,000 killed all, even in clumps. Feldt²² found that for inhibition one part in 5,000 up to one part in 50,000 was needed according to the preparation used. In our own work we found it necessary to use at least one part of copper chloride in 100,000 parts of media to completely inhibit development of the culture. In spite of its low tuberculocidal power, copper has received more attention than the other metals from investigators in the chemotherapy of tuberculosis. As early as 1885, Luton²³ recommended the use of copper in human tuberculosis and to some extent it has been used ever since. It came into renewed prominence in 1912, when Gräfin v. Linden reported before the Tenth International Congress for Tuberculosis that, if treatment of guinea-pigs with copper and methylene blue was begun within fourteen days after inoculation with a tubercle culture of relatively low virulence and continued for six months, the disease was checked or healed in 75 per cent of the cases. The copper was more energetic in its action than the methylene blue. Since 1912, v. Linden²⁴ has published numerous preliminary communications and in 1915, a complete and detailed report of her work. Many other investigators have attempted to verify her work and have failed to obtain any results comparable to hers, while every claim put forward by her has been contradicted by careful investigators. Moewes and Jauer²⁵ used several compounds of copper, among them the preparations recommended by v. Linden and found them uniformly toxic for animals and useless, so far as influencing the progress of the disease is concerned. Corper²⁶ analyzed for copper the tissues of many tuberculous animals treated for long periods with different preparations of copper and found no appreciable amount of copper in the tuberculous areas and observed no effect on the course of the disease.

GOLD.

Basing their experiments on the previously quoted statement of Koch regarding the high inhibitory value of gold salts, reports of experiments on the therapeutic power of gold salts in tuberculosis were published in 1913 and the following years by Bruck and Glück, by Spiess and Feldt, by Mayer²⁰ and others. The work of Bruck and Glück²⁷ is concerned mainly with the action of gold potassium cyanide, with which they obtained good results, especially in lupus. Feldt²⁸ and Spiess and Feldt²⁹ have made and patented under the trade name "Aurocantan" a compound of gold cyanide with ethylene-diamine-cantharidin. The compound used is mono-cantharidin-ethylene-diamine-aurous cyanide. They state that both cantharidin and gold are specific for tuberculosis, that the toxicity of cantharidin is greatly reduced by condensation with ethylene diamine, and that the aurocantan is much less toxic and more effective than the gold cyanide alone. The preparation is administered intravenously and has been used alone and also combined with other treatment; e.g., tuberculin, borcholin, and with ultra violet rays. They consider that the effect of the gold is due largely to its oxygen carrying power. Its effect in tuberculous animals consists, according to the claims of these workers, in increase of weight, prolongation of life, checking the spread of the disease in the internal organs and favoring connective tissue encapsulation of the tubercles. They also claim

that the tubercle bacilli in the body become thin, granular and strongly agglutinated. In patients with open pulmonary tuberculosis, the sputum diminished in amount, the tubercle bacilli disappeared, the amount of sputum protein diminished and physical signs improved. These workers say that, as silver is specific for pyogenic cocci, arsenic for spirochetes and copper for algæ, so is gold specific for tubercle bacilli. I have for some months been carrying on investigations with gold cyanide and gold potassium cyanide in the treatment of experimental tuberculosis in animals, but my work has not yet progressed far enough for me to be willing to either affirm or deny the claims of these investigators.

MERCURY.

So far as I have been able to ascertain, no work on the mercury therapy of experimental tuberculosis has as yet been published, in spite of the fact that this metal stands at the head of the heavy metals in its tuberculocidal power. The high toxicity of the simpler mercury salts has necessitated the making of new preparations less toxic for the animal tissues while still preserving a high parasitocidal and inhibitory power. Many complex organic compounds have been made and tested as to toxicity, distribution and influence on diseases caused by spirochetes and spirilla. Among these, mention may be made especially of numerous so-called mercurialized dyes, made and tested by several investigators, notably Hahn and Kostenbader.³¹ In one group of these dyes, the mercury is bound to nitrogen, oxygen, or some group outside of the ring; in a second group, the mercury is attached by one valence to a carbon atom in the ring, the other valence being joined to oxygen or some other atom or group; in the third group, the mercury connects two rings, both of its valences being bound to carbon. Some of these compounds were made synthetically, others by substitution in the dye molecule. Jacobs and Heidelberger³² have made a number of mercuric acetate derivatives of the aromatic amines, most of their compounds having a dye character. For some months, I have been carrying on investigations on the action of some mercury dye compounds, as well as other mercury salts. My experiments are still in progress and it is too early to give any decisive answer to the question whether mercury can be considered efficient in the treatment of tuberculosis. Most of the preparations used are toxic and have had no apparent beneficial influence on the course of the disease. A few, like mercury nucleide and 1-amino-2(p-naphthalene-azo-phenyl mercuric acetate)5 sulphonic acid have consistently diminished the dissemination of the tuberculous lesions. Tuberculous animals treated with mercurialized methylene blue have lived much longer than untreated controls and the distribution of tubercles in the internal organs has been much less extensive. A complete and detailed report of all these investigations will be published later.

So far, then, our chemotherapeutic studies have resulted in no experimental demonstration of any chemical specifics for tuberculosis. The evidence regarding iodine, arsenic, copper and most of the dyes has been in the main negative. Gold, mercury and certain dye compounds are still on trial and no decisive results have yet been obtained. It must be concluded, therefore, that we are still just at the beginning of the experimental study on the chemotherapy of tuberculosis.

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SUGAR CONTENT OF THE CEREBROSPINAL FLUID IN HEALTH AND DISEASE.*

BY WALTER M. KRAUS, A.M., M.D., AND G. G. CORNEILLE, M.D.,
NEW YORK CITY, N. Y.

THE clinical value of the estimation of the sugar content of the cerebrospinal fluid is still open to discussion. A series of cases were therefore tested with the idea of determining the changes which disease, particularly syphilis of the nervous system, might cause.

In the natural course of events, many cases which were suspected to be syphilitic did not show any evidence of this by biological tests. These cases were, as far as the central nervous system was concerned, normal. The investigations of von Jaksch¹ in twenty cases showed the range of normal to be from .06 to .08 per cent. Kopetsky² used Benedict's copper method and found an average value of .046 per cent in eight cases. Mestrezat³ found the average in twenty cases to lie between .048 and .053.†

Method.—The spinal fluid was examined within a few minutes of withdrawal. The globulin was tested and spinal fluid count and Wassermann were done. Sugar was determined by the Lewis and Benedict⁴ method.

Observation.—Determinations were made fifty times in forty-six cases. These fall into three general groups.

- (1) Normal. Table I. These cases were so considered because there was no evidence of organic involvement of the nervous system.
- (2) Syphilitic disease of the brain, spinal cord and meninges. Table II.
- (3) Purulent meningitis. Table III.

The average of normal cases was .0818 per cent, the range .055 to .110 per cent. The average for syphilitic cases was .0815 per cent, the range .055 to .110 per cent. There was no correspondence between the amount of globulin, the number of cells and the sugar content. Syphilis apparently does not alter the normal sugar content.

Two cases of purulent meningitis were followed daily.

TABLE I. NORMAL CASES.

NO.	DIAGNOSIS.	% SUGAR.	WASSERMANN'S		CELLS.	GLOBULIN.	REMARKS.
			BLOOD	SP. FL.‡			
3	Normal	.060	0	0	0	0	
22	"	.072	0	0	0	0	
39	"	.100	0	0	0	0	
57	"	.090	0	0	0	0	
56	Chr. Card.						
	Valv. Dis.	.100	2	0	0	0	
19	"	.086	0	0	0	0	
41	"	.100	0	0	0	0	
7	Unclassified						
	Psychopathy	.074	3	5	0	0	

*From the Fourth Medical Division and the Psychopathic Department of Bellevue Hospital, New York.

†Read before the Neurological Section of the New York Academy of Medicine, Feb. 8, 1916. Just prior to the reading of this paper, one appeared in The American Journal of the Medical Sciences, by A. H. Hopkins (Vol. cl, No. 6). According to his observations, the normal lies between .06 per cent and .075 per cent. The Bang method was used.

‡=18 units is equivalent to a completely positive reaction.

TABLE I.—NORMAL CASES (CONT'D).

NO.	DIAGNOSIS.	WASSERMANN'S					REMARKS.
		% SUGAR.	BLOOD SP. FL.		CELLS.	GLOBULIN.	
4	Manic Depressive Insanity	.045	0	0	1	0	
27		.110	0	0	1	0	
48		.088	0	0	0	0	
21	Allied to Dementia Precox.	.110	0	0	3	trace	
28		.104	0	0	1	0	
52	Dementia Precox	.100	0	0	0	0	
49	Paranoid State	.095	6	0	0	0	
54	Chronic Alcoholism	.110	0	0	2	0	
25	Acute Enteritis	.070	2	0	0	0	
14	Gas Poisoning	.110	?	0	3	0	
39	Abdominal Tumor	.080	?	?	2	0	
16	Infective Exhaustive Psychosis	.073	15	0	0	0	
10	Gen'l Arteriosclerosis	.065	4	2	1	0	
5	"	.066	0	0	0	0	

TABLE II. SYPHILIS OF THE CENTRAL NERVOUS SYSTEM.

NO.	DIAGNOSIS.	WASSERMANN'S					REMARKS.
		% SUGAR.	BLOOD SP. FL.‡		CELLS.	GLOBULIN.	
6	Gen'l Paralysis	.098	15	18	10	÷÷	
8	"	.110	16	18	14	÷÷	
9	"	.075	18	18	31	÷÷	
23	"	.071	15	15	11	÷÷	
29	"	.072	15	18	72	÷÷	
30	"	.060	10	15	25	÷÷	
38	"	.068	12	15	21	÷÷	
12	"	.085	18	AC	62	÷÷	
53	"	.090	0	0	1	0	
31	"	.072	15	15	12	÷	
50	"	.090	14	15	44	÷	
42	"						
	(Juvenile)	.090	15	15	10	÷	Average Gen'l Paralysis=80
37	Tabes Dorsalis	.055	9	15	198	÷÷	
39	"	.080	?	?	39	÷	
43	"	.090	?	?	39	÷	Average Tabes Dorsalis=75
17	Cerebrospinal Syphilis	.068	14	14	20	÷÷	
40	"	.020	—	15	8	÷	
36	"	.110	6	18	Contaminated	Contaminated	
34	"	.060	17	18	157	÷	
13	"	.082	16	5	2	÷	
20	"	.075	15	15	11	÷	Average Cerebrospinal Syphilis=86

‡=18 units is equivalent to a completely positive reaction.
Ac=Anticomplementary.

TABLE III. PURULENT MENINGITIS.

NO.	DIAGNOSIS.	% SUGAR.	CELLS.	GLOBULIN.	REMARKS.
<i>Case 1.</i>					
2/19	Meningococcus	.045	12,000*	++++	
2/20	"	.050	6,500**	+++	
2/22	"	.055	188	++	
15/23	"	.068	99	++	
24/24	"	.080	?	?	Recovered
<i>Case 2.</i>					
12	Pneumococcus				
	Meningitis	.022	1,300	++	
13	"	.015	9,000	+++	Died

*=87% polymorphonuclears.

**=85% polymorphonuclears.

It will be seen from Table 3 that in the case which recovered the value steadily rose, while in that which died it fell.

The observation that the reduction of Fehling's solution (or Benedict's) is absent, i.e., that sugar is absent in acute purulent meningitis, has not been found to be true, a fact emphasized by Du Bois and Neal,⁵ and Schloss and Schroeder.⁶

The change in the amount of sugar in purulent meningitis is of great value from a prognostic point of view. A rise is favorable and a fall unfavorable. This is emphasized by Hopkins and by a number of others quoted by him as well as by Schloss and Schroeder. The determination in syphilis of the nervous system adds nothing of value.

We wish to express our thanks to Dr. Cyrus J. Strong, Dr. Alexander Lambert and Dr. M. S. Gregory for permission to use cases on their services.

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AN ARTIFICIAL RESPIRATION APPARATUS FOR GENERAL LABORATORY PURPOSES *

BY PAUL J. HANZLIK, M.D., CLEVELAND, OHIO.

THERE are many kinds of simple and elaborate apparatuses for securing artificial respiration. It would seem, therefore, superfluous and unnecessary to add to the list. However, as a rule, these are not suitable for the use of large classes of students. Many are intended for a single animal, or at most two or three. If suitable for more than one animal the rate and volume of respiration cannot be altered for any particular animal, since the mechanism provides for no individual variation. This is inconvenient and undesirable, especially in teaching in laboratories where animals in any lot may differ very widely in size, and the respiratory requirements for different experiments may differ considerably.

For these and other reasons, it seemed desirable to devise an apparatus which would obviate these difficulties; and so as to be generally serviceable for both teaching and research. At the same time, such an apparatus should be simple, and reasonable in price. It is believed that these requirements have been met to a large extent by an apparatus which has been devised and is now in successful operation in this laboratory.

The apparatus is built on a unit plan system, and consists briefly of a long, motor-driven shaft which in turn drives a series of pumps by a set of pulleys which permit the regulation of the rate and volume of respiration. Each pump and fittings properly attached to a table constitute a unit, and these may be multiplied up to the driving capacity of the motor. Each table is detachable, the apparatus is practically out of view and can occupy what might be termed the "dead-space" in the laboratory, that is, the main shaft is attached to the floor alongside the wall, and the pumps are situated underneath the table tops. Fig. 1 illustrates all of the attachments necessary in the makeup of a unit, with the exception of the motor. Figs. 2 and 3 illustrate in detail the parts used in the construction of several portions of the apparatus. These and other parts may now be described.

Motor.—The size will be determined by the number of units it is desired to put into operation. The exact horsepower to operate a single unit has not been determined, but a $\frac{1}{16}$ Hp. motor will operate two units, and we have five units now in operation by a $\frac{1}{4}$ Hp. motor. It is safe to assume that a $\frac{1}{4}$ Hp. motor can easily operate 8 to 10 units. A worm attached to the motor shaft operates a cog-wheel on the main shaft, which rotates 26 times per minute. The speed of the motor was reduced by a specially cast cog-wheel. The motor had 700 r.p.m. In order to obtain 26 r.p.m., on the main shaft, a cog-wheel with 24 teeth was cast. (Theoretically, this wheel should give 29.1 r.p.m.) In order to reduce friction and noise as much as possible, the gears are immersed in an oil bath. It may be mentioned here that the motor is the most expensive part of the apparatus. However, this can be considerably mitigated by securing a used motor, which for ordinary laboratory purposes will serve very well.

Main Shaft.—This consists of an iron rod $1\frac{1}{16}$ inch in diameter, running through hangers, screwed to the floor close to the wall. A 30-foot length is advisable for 5 units. This secures plenty of working space between tables.

*From the Pharmacological Laboratory, Western Reserve University, Cleveland.

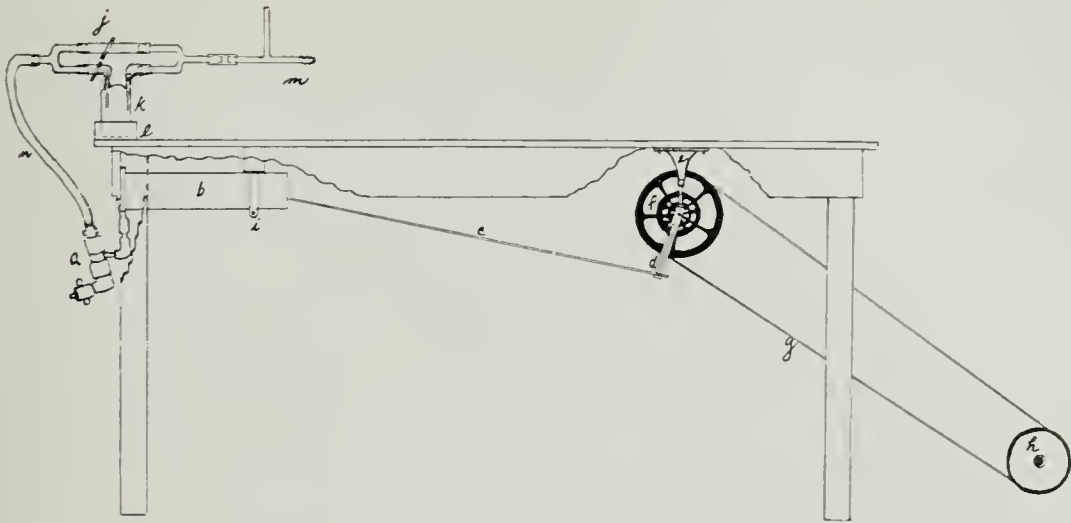


Fig. 1.—Artificial respiration unit (sectional view, scale about 1 to 20).

- a. valve.
- b. cylinder (pump).
- c. piston shaft.
- d. slit crank.
- e. adjustable hanger.
- f. iron grooved pulleys.
- g. round belt.
- h. wooden grooved pulley (with cross section of main shaft in center).
- i. brass collar for holding pump.
- j. anesthetizing attachment (Y-pieces placed upright; natural position horizontal).
- k. Woulff bottle.
- l. drilled wooden block
- m. tracheal T-cannula.
- n. rubber tubing connection.

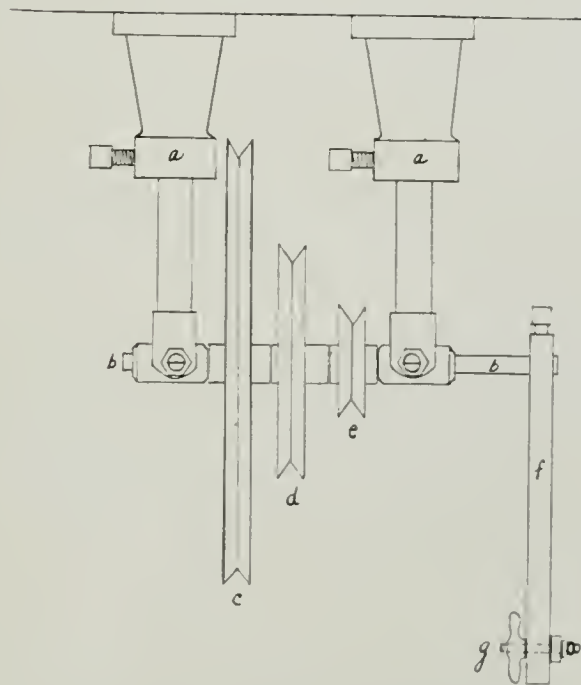


Fig. 2.—Adjustable hangers with iron grooved pulleys and slit crank (front view, scale about 1 to 4.6).

- a. adjustable hanger ($5\frac{1}{2}$ inch).
- b. iron shaft ($\frac{1}{2} \times 8$ inches).
- c. 8-inch iron grooved pulley.
- d. 4-inch iron grooved pulley.
- e. 2-inch iron grooved pulley.
- f. slit crank.
- g. set-screw attachment.

Wooden-Grooved Pulleys.—Their diameter is 6 inches, and width 2 inches. These are attached to the main shaft at intervals of 6 feet, and possess a groove about $\frac{5}{16}$ inch in depth for a round belt, which drives the set of pulleys underneath the table. The length of the belt will depend on the proximity of the table to the main shaft. Patent snap-hooks for joining the belt are most convenient.

Iron-Grooved Pulleys.—Three are suspended by means of a small iron shaft ($\frac{1}{2} \times 8$ inches) in a pair of adjustable hangers ($5\frac{1}{2}$ inch) screwed underneath the table top. (1) The 8 inch (diameter) wheel gives 20 revolutions, that is, a respiratory rate of 20 per minute; (2) The 4 inch wheel gives a respiratory rate of 40 per minute; (3) The 2 inch wheel gives a respiratory rate of 82 per minute, and is practically suitable for insufflation. The small shaft extends out sufficiently for the attachment of a brass slit-crank, to which is attached a piston shaft. Fig. 2 shows a front view of the pulleys with hangers and slit-crank.

Slit-Crank.—The crank is $6\frac{1}{2}$ inches long, $\frac{3}{4}$ inch wide and $\frac{3}{8}$ inch thick, with a slit in the middle, extending the full length of the crank, except for the closed ends. Shifting the piston shaft up and down the slit provides for almost any desired change in volume of respiration, ranging from 240 c.c. to 1,250 c.c. in our standard pump. A set-screw attachment on the piston shaft permits an adjustment of the respiratory volume at a moment's notice by simply relaxing the belt, which is easily and readily secured by merely pushing the table in the direction of the main shaft. By means of this slit crank, and a suitably regulated T-tube in the tube leading to the trachea, we have successfully respired animals ranging from a small (250 gm.) guinea-pig to a 20 Kg. dog with the same pump.

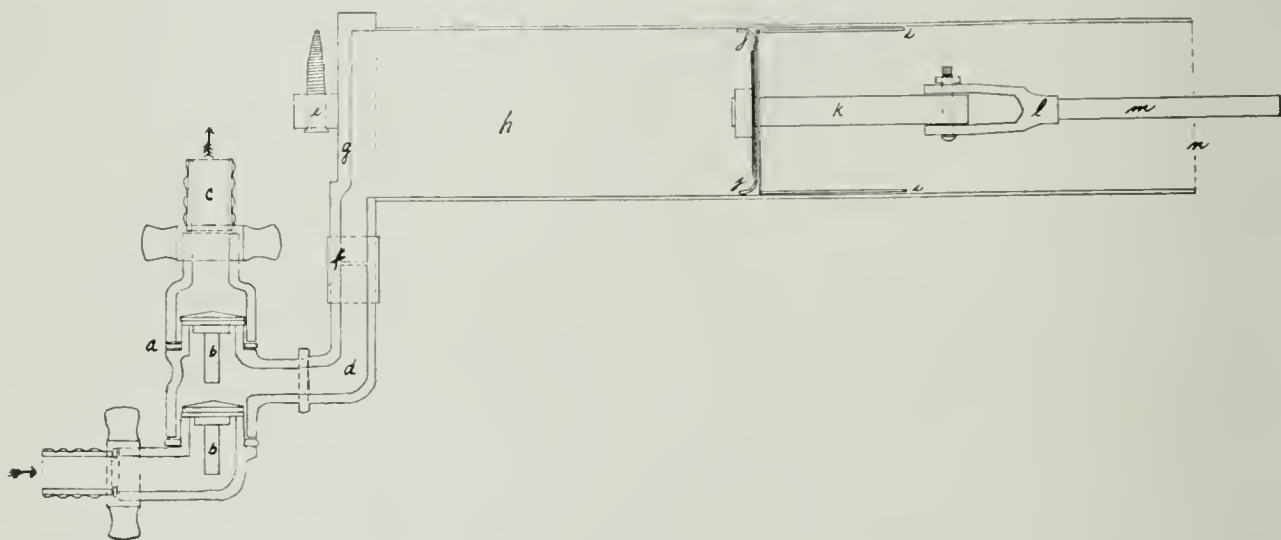


Fig. 3.—Pump and valve (sectional view, scale about 1 to 4.6).

- a. valve.
- b. brass pin.
- c. air outlet, and rubber tubing attachment.
- d. iron elbow.
- e. projection with screw for fastening pump to front end of table.
- f. brass union.
- g. patent brass disc (distal end of cylinder).
- h. cylinder.
- i. piston-cup.
- j. leather washer.
- k. short piston tube.
- l. fork attachment to piston shaft.
- m. piston shaft.
- n. open proximal end of cylinder.

Piston-Shaft.—This consists of a brass tube 40 inches long and $\frac{3}{8}$ inch in diameter, with a set screw attachment to the slit-crank, and a fork attachment to the piston proper within the cylinder of the pump (Fig. 3).

Cylinder.—This consists of a brass tube 15 inches long and 3 inches in diameter, closed at the distal end with a patent brass disc, provided with a projection in the center for screwing to the table, and an outlet which communicates with the inside of the cylinder for the escape of air. The proximal end of the cylinder is left open to permit freer operation of the piston-shaft. The capacity of such a cylinder is 1,750 c.c. and this is our standard size. It is easily operated and answers for all practical purposes to which it may be put in artificial respiration. However, the entire capacity is not utilized, because a relatively

long piston-cup, which permits easy working of the piston attachments with the minimum of friction, is used.

Piston.—This is illustrated in Fig. 3, and consists of a brass cup 3 inches deep and $2\frac{7}{8}$ inches in diameter, with a short stout brass tube $4 \times \frac{1}{2}$ inches attached to the center of the bottom, to which is fitted a leather washer.

Valve.—The structure of this is sufficiently illustrated in Fig. 3, and simply consists of a chamber enclosing two short brass pins with their leather bases pointing upward, so that air is permitted to escape in one direction only. The valve is directly attached to the distal end of the cylinder by means of ordinary iron elbows used in joining gas or water pipes. This valve functionates in the upright or slightly oblique position only. Suitable ends on the valves are provided for the attachment of rubber tubing for the conduction of air through an anesthetizing attachment.

Anesthetizing Attachment (Fig. 1).—This consists of an ordinary Woulff bottle with two necks. By means of Y-pieces attached to the necks, ether or air can be passed at will by the regulation of screw stop-cocks placed on the rubber connections. The proximal Y-piece connects by means of a rubber tube ($\frac{1}{2}$ inch in diameter) with the valve. One limb of the Y-piece connects with a neck of the Woulff bottle, the other limb connects with a corresponding limb of the distal Y-piece, the other limb of which in turn connects with the other neck of the bottle. The distal Y-piece is now joined by means of rubber tubing to a T-piece, the distal end of which can serve as a tracheal cannula.

The Woulff bottle is easily secured in place by inserting it into a drilled wooden block permanently fastened to the corner of the table and situated close to the pump. The anesthetizing attachment permits of a considerable amount of motion, and all of its parts are easily detachable.

All of the attachments, with the exception of the piston cup and slit-crank, which have been described, are obtainable in the market. The valves and cylinders are obtainable from the Cleveland Faucet Company. The adjustable hanger and iron grooved pulleys can be secured from T. F. Welch & Co., Boston. The piston cup can easily be made by a mechanic of ordinary skill, and a slit-crank is most conveniently secured by having it specially cast.*

The advantages claimed for this apparatus may be summarized as follows:

It is easily constructed, because all parts are easily obtainable in the market; it operates practically automatically; is easily adjustable; is serviceable for several different-sized animals at the same time; is relatively inexpensive and durable.

My thanks are due to our technician, Mr. Morris Dan, for aid in the construction of the apparatus, and to Mr. C. J. West, of the Second Year Class, for the drawings.

*The cost prices of the various parts and attachments for a unit are approximately as follows: Adjustable hanger, 90c; iron-grooved pulleys, 8-inch, 90c, 4-inch, 75c, 2-inch, 50c; slit-crank, 7c; piston-shaft, 15c; fork for piston-shaft, 5c; pump, including cylinder, piston and valve, \$10.00; belting (5 ft.), 35c; accessories, about \$1.25—total, about, \$15.00. For the following articles used to operate five units, the cost was as follows: Used $\frac{1}{4}$ Hp. motor, \$10.00; 30-ft. shaft, \$3.00; 7 hangers and couplings for shaft at \$1.50 per pair, \$10.50; 5 wooden-grooved pulleys, at \$1.00=\$5.00; accessories, about \$5.00. The total cost to set 5 units into operation was about \$117.00.

THE COLOR REACTIONS OF PROTEINS AND THEIR SPLIT PRODUCTS *

BY HERBERT W. EMERSON, M.D., AND JOHN S. CHAMBERS, M.S.,
ANN ARBOR, MICH.

THE following experiments on the color reactions of some proteins and their split products were performed, with the hope that they might throw more light on the way in which the protein molecule is cleaved by Vaughan's method.¹ The variation in the intensity of the color reaction is expressed by varying the number of + signs in tabulating the results, and also, when possible, by determining the highest dilution that will give a positive color reaction.

TABLE I.

The Biuret test carried out in the usual way gives the following results:

Dilutions.	1-1000.	1-5000.
Casein	+ + +	+
Casein Poison	+ +	+
Casein Residue	± (1-100 +)	—
T. B. C. Germ Substance	+ + +	±
T. B. C. Poison	+ + +	+
T. B. C. Residue	+	—
Colon Germ Substance	+ + +	±
Colon Poison	+ + +	+
Colon Residue	± (1-100 +)	—
Pepton	+ +	—
Egg White	+ + +	+
Dog Liver Poison	+ + +	±

TABLE II.

Gies's² modification of the Biuret test gives practically the same results as the Biuret.

Dilutions.	1-1000.	1-5000.
Casein	+ + +	+
Casein Poison	+ + +	—
Casein Residue	— (1-100+)	—
T. B. C. Germ Substance	+ + +	±
T. B. C. Poison	+ + +	+
T. B. C. Residue	+	—
Colon Germ Substance	+ + +	±
Colon Poison	+ + +	+
Colon Residue	+	—
Pepton	+ +	—
Egg White	+ + +	+
Dog Liver Poison	+ + +	—

TABLE III.

The Xanthoproteic test carried out in the usual manner gives results as follows:

Dilutions.	1-1000.	1-5000.
Casein	+ + + + +	+ +
Casein Poison	+ + + +	+
Casein Residue	+	—
T. B. C. Germ Substance	+ + +	—

*From the Hygienic Laboratory of the University of Michigan.

TABLE III (CONT'D).

Dilutions.	1-1000.	1-5000.
T. B. C. Poison	+ + + +	±
T. B. C. Residue	+ + +	—
Colon Germ Substance	+ + +	—
Colon Poison	+ + + +	±
Colon Residue	+ + +	—
Pepton	+	—
Egg White	+ + + +	+ +
Dog Liver Poison	+ + +	+

TABLE IV.

Millon's test gave results as follows:

Dilutions.	1-1000.	1-5000.
Casein	+ +	±
Casein Poison	+ + +	+
Casein Residue	(Powder —)	—
T. B. C. Germ Substance	+ +	—
T. B. C. Poison	+ + +	+
T. B. C. Residue	(Powder —)	—
Colon Germ Substance	+ + + +	+
Colon Poison	+ + + + +	+ + +
Colon Residue	(Powder —)	—
Pepton	+	—
Egg White	+ +	—
Dog Liver Poison	+ + +	+

TABLE V.

Bardach's^{3, 4} iodoform crystal test, in which the presence of proteins interferes with the formation of typical iodoform crystals, was carried out with very concentrated solutions of the proteins and the split products with the following results:

Casein	+
Casein Poison	+
Casein Residue	—
T. B. C. Germ Substance	+
T. B. C. Poison	+
T. B. C. Residue	—
Colon Germ Substance	+
Colon Poison	+
Colon Residue	—
Pepton	+
Egg White	+
Dog Liver Poison	+

TABLE VI.

The Molisch test, using only very concentrated solutions of the proteins and the split products, gives results as follows:

Casein	+ ?
Casein Poison	—
Casein Residue	+ + +
T. B. C. Germ Substance	+ +
T. B. C. Poison	—
T. B. C. Residue	+ + +
Colon Germ Substance	+ +
Colon Poison	—
Colon Residue	+ + + +
Pepton	+
Egg White	+
Dog Liver Poison	—

TABLE VII.

The Adamkiewicz reaction, using glacial acetic acid and concentrated sulphuric acid in the proportion 2:1 to which the dry powdered substance to be tested is added, gave the following results:

Tyrosin*	+ a pinkish violet color,
Tryptophane*	+ — + a dark yellowish red,
Casein	+ + light pinkish straw color,
Casein Poison	+ + dark brownish red,
Casein Residue	+ straw color with pink tinge,
T. B. C. Germ Substance	+ + dark deep red,
T. B. C. Poison	+ + brownish red,
T. B. C. Residue	+ + + very deep red,
Colon Germ Substance	+ + + + very deep red,
Colon Poison	+ + + dark brownish red,
Colon Residue	+ + + dark brownish red,
Pepton	+ + light straw pink,
Egg White	+ + + deep violet red,
Dog Liver Poison	+ + dark brownish red.

TABLE VIII.

The Hopkins Cole³ reaction was carried out by adding a small amount of the powdered substance to be tested to three or four cubic centimeters of the Hopkins Cole reagent. Sulphuric acid was then run down the side of the test tube so as to form two layers.

Casein	+ + + pink ring,
Casein Poison	? yellowish brown cloudy ring,
Casein Residue	+ slight pink ring,
T. B. C. Germ Substance	+ + pinkish violet ring,
T. B. C. Poison	? yellowish brown cloud,
T. B. C. Residue	+ pink violet ring,
Colon Germ Substance	+ + + violet ring,
Colon Poison	? yellowish brown cloud,
Colon Residue	+ light pink ring,
Pepton	+ + + reddish violet ring,
Egg White	+ + + violet ring,
Dog Liver Poison	? yellowish brown cloudy ring, no red or violet.

TABLE IX.

The tests in Table VIII were repeated, adding one drop of furfural to each tube and thoroughly mixing its contents just before the sulphuric acid was run in.

Casein	+ + + + dark yellowish red ring,
Casein Poison	+ + + + " " "
Casein Residue	+ very slight yellowish pink ring,
T. B. C. Germ Substance	+ + light yellowish red ring,
T. B. C. Poison	+ + + " " "
T. B. C. Residue	+ + + + heavy dark yellowish red ring,
Colon Germ Substance	+ + + + + " " "
Colon Poison	+ + + " " "
Colon Residue	+ + + " " "
Pepton	+ faint pinkish ring,
Egg White	+ + + + yellowish violet ring,
Dog Liver Poison	+ + + + " red "
Tryptophane	+ + + + + " " "
Tyrosin	+ very slight pinkish straw colored ring.

TABLE X.

Benedict's modification of the Hopkins Cole reaction gave the same results as is shown by the following:

Casein	+ + + reddish pink ring,
Casein Poison	? brown cloudy ring, no red,

*Controls were run by adding one drop of a one per cent solution of tyrosine or tryptophane and one drop of furfural to the acid mixture.

TABLE X (CONT'D).

Casein Residue	+ pink ring,
T. B. C. Germ Substance	+ + violet ring,
T. B. C. Poison	? brown cloudy ring, no red,
T. B. C. Residue	+ + pink ring,
Colon Germ Substance	+ + pink ring,
Colon Poison	? light brown cloudy ring, no red,
Colon Residue	+ pink ring,
Pepton	+ + + bluish violet ring,
Egg White	+ + + reddish violet ring,
Dog Liver Poison	? brown cloudy ring, no red.

TABLE XI.

The tests in Table x were repeated and modified by adding one drop of furfural to each tube and thoroughly mixing just before the sulphuric acid was run in. The following results were obtained:

Casein	+ + + dark yellowish red ring.
Casein Poison	+ + + " " " "
Casein Residue	+ very slight yellowish pink ring.
T. B. C. Germ Substance	+ + light yellowish red ring,
T. B. C. Poison	+ + " " " "
T. B. C. Residue	+ + + heavy dark red ring with yellowish tinge.
Colon Germ Substance	+ + + + yellowish red ring,
Colon Poison	+ + + " " "
Colon Residue	+ + + " " "
Pepton	+ + faint pinkish ring,
Egg White	+ + + + yellowish violet ring,
Dog Liver Poison	+ + + + yellowish red ring,
Tyrosine	+ very slight pinkish straw colored ring,
Tryptophane	+ + + + + yellowish red ring.

TABLE XII.

The Acree Rosenheim formaldehyde test was carried out by adding two or three drops of a 1-5000 formaldehyde solution to about four cubic centimeters of strong aqueous solution of the substances to be tested and thoroughly mixing before running in the sulphuric acid.

Casein	+ + violet ring,
Casein Poison	? brown cloudy ring, no red.
Casein Residue	+ pink ring,
T. B. C. Germ Substance	+ faint violet ring,
T. B. C. Poison	? brown cloudy ring, no red,
T. B. C. Residue	+ + light pink ring,
Colon Germ Substance	+ + pink ring,
Colon Poison	? brown cloudy ring, no red,
Colon Residue	+ + pink ring,
Pepton	+ + + bluish violet ring,
Egg White	+ + reddish violet ring,
Dog Liver Poison	? brown cloudy ring, no red.

TABLE XIII.

The tests in Table xii were repeated and modified by adding one drop of furfural to each tube and thoroughly mixing before the sulphuric acid was run in. The results obtained were as follows:

Casein	+ + + yellowish red ring,
Casein Poison	+ + + " " "
Casein Residue	+ + pink ring,
T. B. C. Germ Substance	+ + yellowish red ring.
T. B. C. Poison	+ + " " "
T. B. C. Residue	+ + + " " "
Colon Germ Substance	+ + " " "

TABLE XIII (CONT'D).

Colon Poison	+ + yellowish red ring,
Colon Residue	+ + " " "
Pepton	+ faint pink ring,
Egg White	+ + brownish red ring,
Dog Liver Poison	+ + yellowish red ring.

TABLE XIV.

The benzaldehyde test, in which the development of a green to a blue color indicates the presence of tryptophane, was carried out as follows: equal volumes of concentrated hydrochloric acid and a two or three per cent solution of the substances to be tested were mixed in a test tube, one drop of ferric chloride and two drops benzaldehyde were added and the contents heated to boiling.

Casein	+ + + + intense blue color,
Casein Poison	— black oily residue, no blue or green color,
Casein Residue	+ light yellowish green,
T. B. C. Germ Substance	+ + + + sky blue,
T. B. C. Poison	— black oily residue, no blue or green color,
T. B. C. Residue	+ dark bluish green,
Colon Germ Substance	+ + + light blue with yellow tinge,
Colon Poison	— brown liquid, oily residue, no blue or green,
Colon Residue	+ + dark brownish green,
Pepton	+ brown precipitate with green tinge,
Egg White	+ + + intense sky blue,
Dog Liver Poison	— black oily residue, no green or blue.

SUMMARY.

1. The proteins and their split products, the protein poisons and the residues, all give the biuret reaction. The residues will not give the reaction in dilutions much greater than (1-100). This indicates that the proteins and their split products contain an acid amide group and other substituted amide groups attached to neighboring carbon atoms, and that the end products have not been deamidized in cleaving.

2. Gies' biuret reagent gives similar results.

3. The proteins and their split products all give the xanthoproteic reaction and the poisons in greater dilutions than the proteins or the residues, indicating that they all contain benzene nuclei and that in cleaving these tend to concentrate in the poison.

4. The residues do not give Millon's reaction and the poisons give it in greater dilutions than the proteins indicating that all of the mono-hydroxy-benzene nuclei (tyrosine) are cleaved off into the poison. It is interesting to note the strong reaction given by the colon cell substance and by the poison.

5. The residues do not give Bardach's reaction and all soluble proteins are said to give it. The residues are alkaline and soluble.

6. The poisons do not give the Molisch reaction and the residues give a stronger Molisch than the proteins indicating that the carbohydrate groups are cleaved off into the residues. Casein gives a very weak Molisch reaction and casein residue a strong reaction, this may be due to tryptophane, but we think not, as the casein residue gives only a weak tryptophane reaction. Casein residue does not reduce Fehling's solution even after boiling with dilute hydrochloric acid. Part of the residue is insoluble in 10 per cent alkali and this insoluble part gives the Molisch reaction and also tests for nitrogen.

TABLE XV

COLOR REACTION	CASEIN	CASEIN POISON	CASEIN RESIDUE	E. B. C. GERM SUBSTANCE	T. B. C. POISON	T. B. C. RESIDUE	COLON GERM SUBSTANCE	COLON POISON	COLON RESIDUE	PEPTONI	EGG WHITE	DOG LIVER POISON
Biret.....	(1-5000) +	(1-5000) +	(1-100) +	(1-5000) ±	(1-5000) +	(1-1000) +	(1-5000) ±	(1-5000) +	(1-100) +	(1-1000) ++	(1-5000) +	(1-5000) ±
Gies' Biret.....	(1-5000) ++	(1-1000) +++	(1-100) +	(1-5000) ±	(1-5000) +	(1-1000) +	(1-5000) ±	(1-5000) +	(1-1000) +	(1-1000) ++	(1-5000) +	(1-1000) ++
Xanthoproteic.....	(1-5000) ++	(1-5000) +	(1-1000) +	(1-1000) +++	(1-5000) ±	(1-1000) +++	(1-1000) +++	(1-5000) ±	(1-1000) +++	(1-5000) +	(1-5000) ++	(1-5000) +
Millon's	(1-5000) ±	(1-5000) +	Powder —	(1-1000) ++	(1-5000) +	Powder —	(1-5000) +	(1-5000) +++	Powder —	(1-1000) +	(1-1000) ++	(1-5000) +
Bardach.....	+	+	—	+	+	—	+	+	—	+	+	+
Molisch.....	—	—	+	++	—	+++	++	—	+++	+	+	—
Adamkiewicz.....	++	++	+	++	++	+++	+++	+++	+++	++	+++	++
Hopkins Cole.....	+++	?	+	++	?	+	+++	?	+	+++	+++	?
Hopkins Cole & Furfural.....	++++	++++	+	++	+++	++++	++++	+++	+++	+	++++	+
Benedict's Hopkins Cole.....	+++	?	+	++	?	++	++	?	+	+++	+++	?
Benedict's Hopkins Cole & Furfural.....	++++	++++	+	++	+++	++++	++++	+++	+++	++	++++	++++
Acree Rosenheim Formaldehyde Test.....	++	?	+	+	?	++	++	?	++	+++	++	?
Acree Rosenheim Test + Furfural.....	+++	+++	++	++	++	+++	++	++	++	+	++	++
Reichl's Benzaldehyde Test	++++	—	+	++++	—	+	++++	—	++	+	++++	—

7. The proteins and their split products all give the Adamkiewicz reaction indicating that they all contain the tryptophane group. Solutions of tyrosine give pinkish violet colors which are similar to the tryptophane reactions in dilute solutions.

8. The protein poisons do not give good Hopkins Cole reactions when performed in the regular way. The proteins and residues do. They all give good positive reactions when furfural is added. The protein poisons probably give negative reactions because they contain no carbohydrate.

9. Benedict's modification of Hopkins Cole reagent gives similar results both with and without furfural.

10. The Acree Rosenheim formaldehyde test gives similar results to those obtained with the Hopkins Cole reagent both with and without furfural.

11. The proteins and the residues give positive results with the benzaldehyde test (Reichl's reaction). The poisons give negative reactions and the addition of furfural has no effect.

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LABORATORY METHODS

PHYSICAL CHARACTERISTICS OF SPINAL FLUID IN VARIOUS DISEASES*

BY A. LEVINSON, M.D., CHICAGO, ILL.

WITH the advance of our knowledge of the chemistry of spinal fluid, we are gradually losing sight of the physical characteristics of the fluid. This is unfortunate, as the physical properties are often valuable in establishing the diagnosis and even the prognosis of the disease, sometimes being as reliable as a chemical test. I should like to point out a few of these characteristics that seem to be of clinical importance.

APPEARANCE OF FLUID IMMEDIATELY AFTER WITHDRAWAL FROM THE BODY.

It is well known that normal fluid is clear and colorless and that meningitic fluid is turbid in appearance. It is not well known, however, that different types of meningitis impart a rather characteristic color to the fluid. Repeated observations have convinced me that while the color differs with the consistency of the fluid which in turn is in constant ratio to the stage of the disease, there is a characteristic tinge that is present in most cases in all stages of the disease unless altered by serum.

The fluid in epidemic meningitis is yellowish in color, with a greenish tinge in it. The more advanced the disease, the more yellow the color of the fluid. At times the fluid will turn green of its own accord. This has happened in a number of our cases (when no contamination has taken place).

When a sediment forms in the meningococcic variety the supernatant fluid still remains yellowish in color.

The fluid in pneumococcus meningitis is pearl gray in color. Even after the sediment has formed, the supernatant fluid remains grayish in color. When serum is administered the fluid takes on a yellowish tinge, but not as marked as in the epidemic variety.

In tuberculous meningitis the fluid is colorless, even when it is turbid in consistency it remains colorless.

SEDIMENT.

It is a well known fact that the fluid of tuberculous meningitis forms a pellicle on standing. Lichtheim described this years ago. It is not so generally known, however, that a pellicle or rather a sediment makes its appearance in every form of meningitis. In fifty cases, that I observed with this in view, I noted the formation of a sediment in practically every case of meningitis. Furthermore, each form of meningitis shows a sediment peculiar to its type, which

*From the Sarah Morris Hospital for Diseases of Children and from The Nelson Morris Institute for Medical Research, Chicago, Ill.

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if properly observed may become helpful in establishing the direct as well as the differential diagnosis.

The sediment formation makes its appearance within not later than twenty-four hours. In meningococcic and pneumococcic meningitis, however, the pellicle or sediment forms quite rapidly, usually as early as one-half hour after the removal of the spinal fluid from the body or even earlier. The sediment is likely to appear under any conditions; however, the conditions most favorable for its formation and preservation, are the following:

1. The spinal fluid should be left undisturbed.
2. The tube containing the fluid should be tightly corked. The latter precaution is not as essential as the former.

In order to facilitate the formation of a pellicle, and in order to obtain uniform results with the other tests on the spinal fluid, it is necessary to have a uniform system in the taking of the fluid. For this purpose I should strongly recommend the adoption of the following plan which I have treated more fully elsewhere (*Medical Record*, lxxxix, No. 17). I shall here mention merely the essential features of the plan.

Four sterile and chemically clean test tubes of uniform size are taken, though in most cases three are sufficient for complete examination. The fourth, however, comes in handy if the quantity of fluid is very large. The tubes should be labeled, 1, 2, 3, 4, and should preferably be calibrated.

Tube 1 should receive the first 2 c.c. of fluid, and this amount should be used for.

1. Cytological examination.
2. Direct uncentrifuged smear for microorganisms.
3. One-half c.c. should be used for culture.
4. One c.c. should be used for the permanganate test (the index for organic substances in the spinal fluid). If not examined immediately this test tube and its contents should be placed in the incubator.

Into the second tube should be run from 3 to 5 c.c. of spinal fluid, depending upon the amount and the pressure of the fluid although 5 is preferable. This should be put into a tube rack, and placed in the refrigerator for the formation of a pellicle or sediment, care being taken that the tube is not disturbed. It is best to use a regular cork instead of a cotton plug. This will not only facilitate the formation of a pellicle, but will also preserve the fluid better than will the cotton plug.

Into the third tube should be run from 5 to 8 c.c. of fluid to be used for

1. The second portion of the permanganate test.
2. The different globulin tests (Noguchi, Ross Jones and Nonne).
3. Lange.
4. Wassermann.
5. Sugar contents.
6. Ten c.c. for centrifugation, for examination of the organism if the first portion did not show it.
7. All other tests that one may desire to perform. Into the fourth tube should be run the rest of the spinal fluid desired either for examination or for

the purpose of relieving pressure which is from 5 to 15 c.c. on the average if the case is one of meningitis.

TYPES OF SEDIMENT.

I. The spinal fluid of meningococcus-meningitis is turbid on withdrawal resembling the color of very rich cream. A sediment or pellicle forms in from one-half hour to one and one-half hours later depending upon the stage of the disease.

(a) In the early stages the pellicle is made up of a yellowish white network, the reticuli being close together forming an opaque layer. The upper portion of the network is balloon or dome-shaped, and the base or lower portion is flattened in appearance; the whole network resembling a small bursa. The network usually does not reach the upper level of the fluid, and as a rule, it inclines toward one side of the tube. The pellicle differs from the one formed in tuberculous meningitis in the following particulars:

1. The pellicle is not suspended in the center.
2. There are no processes projecting from the side of the pellicle.
3. The color is more yellowish and the network more firm.
4. The fluid remains serum-like in color and consistency after the formation of the sediment.

(b) In the more advanced stage of meningitis as when the disease has progressed for a number of days, the turbidity upon withdrawal may or may not be marked, but the sediment formation is firmer, resembling a membrane in its appearance. It usually spreads out along one side of the tube, in the form of a heavy film (Fig. B).

(c) In still more advanced cases, there is a sediment that sinks to the bottom, but still as a rule, no coagulation occurs. In this stage a deposit of yellow granules is seen, attached to one side of the tube. These granules may be considered as a manifestation of an advanced stage (Fig. C).

(d) In the very grave cases the sediment is thick and falls by gravity to the bottom. In the cases where this happened and when the pellicle was more than three-fourths inch in height, the prognosis as a rule has been grave (Fig. D).

When the meningitis recedes, the pellicle or sediment takes on a character the reverse of the one that appeared at its formation, often changing from membranous deposit back to a reticular formation (Fig. E).

II. In pneumococcic meningitis, the fluid is usually not so turbid as in meningococcic, and it is grayish in color, a pellicle or sediment forming in one-half hour or more.

(a) In the early stage a pellicle forms which is triangular in shape and hangs suspended in the center of the tube. The pellicle which is grayish in color is firm and made up of fibrous masses. It differs from the T. B. pellicle in that it is more opaque, firmer, and more grayish in color. It differs from meningococcic sediment in that it is not as flattened, and is more firm than the latter.

(b) In the advanced stages the sediment sinks to the bottom in a homogeneous mass. Within five or six hours, as a rule, the sediment coagulates and

takes on the appearance of the boiled white of an egg (Figs. F and G), around the center of a noncoagulated mass.

III. T. B. meningitis forms a pellicle in from six to twelve hours either in the form of a white streak with small projections from the sides, or in advanced cases, in the shape of a fine, triangular-formed network, with thin reticuli, the apex of the sediment touching the surface of the fluid, and the base touching the bottom (Fig. H). The structure is not firm and rough handling will break it or cause it to curl up at the bottom of the tube. The pellicle is always snow-white in color, unless there is a mixture of blood in the tube, and is made up of fine reticuli. The rest of the fluid is clear, even if before the appearance of the sediment it appeared to be slightly turbid.

Often when a T. B. meningitis is mistaken for other forms of meningitis



A B C D E F G H

PELLICLE FORMATION IN MENINGITIS.

- A. Normal.
- B. Meningococcic meningitis, fairly early.
- C. Meningococcic meningitis, advanced (sulphur-like granules).
- D. Meningococcic meningitis, late in disease.
- E. Meningococcic meningitis, severe, on road to recovery.
- F. Pneumococcic meningitis, fatal.
- G. Pneumococcic meningitis, fatal.
- H. Tuberculous meningitis.

and serum is administered the pellicle will still form if the fluid has been left undisturbed. The pellicle formation is of diagnostic value in such cases, since the serum injection renders all chemical tests valueless.

The pellicle formation is of great assistance diagnostically when the spinal

fluid withdrawn is mixed with blood, and all other tests but the bacteriological are thereby rendered useless. The sediment appears in a short time directly above the blood that has settled at the bottom, showing the same characteristics as if no blood were present.

I made an attempt to study the microscopic appearance of the sediment in the various forms of meningitis. The superficial examination of the sediment in meningococcic meningitis showed leucocytes and reticuli. In one case of pneumococcic meningitis the microscopical examination showed fibrinous masses, in another homogeneous masses. I can at present say nothing positive, however, about the microscopic character of the sediment. I hope to be able to go into the investigation more thoroughly in the near future.

FOAM.

I observed a rather peculiar thing in the specimens of spinal fluid that came under my observation—a phenomenon to which I have not yet been able to find any reference in literature—namely the formation of a foam on the surface of pathological specimens of spinal fluid. With one exception I found that all the pathological fluids, covering every form of meningitis and of lues, produced a foam when the tube was shaken even slightly, whereas in the case of normal fluids even the most vigorous shaking did not result in the formation of a thick foam, the utmost being the rise of a thin layer of bubbles to the surface of the fluid. The foam produced in the pathological fluids I found to be from $\frac{1}{2}$ to 2 inches in height, resembling a soap foam in all its details. The foam thus formed persists from one-half to several hours, when left undisturbed, while the bubbles produced in the normal spinal fluid disappear in a very short while. What adds greater significance to the foam formation is the fact that the alkalinity of the spinal fluid that foamed was increased, upon standing, from one-half to one and one-half c.c. of a $n/100$ H_2SO_4 , above the alkalinity obtained right after the withdrawal of the fluid from the body. (The indicator I used was methyl red which I described more fully in the *Archives of Pediatrics*, April, 1916.) The fluid that did not foam showed no increase in alkalinity upon standing. Whether this will hold good in every case is something that remains for investigators in this field to determine. If it does, it may serve as a rough index for pathological fluids.

SUMMARY.

1. The color of the fluid varies with the form of meningitis.
2. A sediment appears in every form of meningitis.
3. The sediment forms most rapidly when the fluid is left undisturbed.
4. The sediment appears in $\frac{1}{2}$ to 12 hours after withdrawal from the body, forming most quickly in meningococcic and pneumococcic meningitis.
5. In meningococcic meningitis the sediment appears at first in the shape of a balloon-like mist, and later takes on a film-like form.

It appears attached to one side of the tube, as a rule, not reaching the surface of the fluid. As the disease advances the sediment drops to the bottom.

6. Pneumococcic meningitis gives a grayish white pellicle triangular in form in the early stages of the disease and a sediment that sinks to the bottom in

the advanced stage. In the latter case the sediment coagulates in a short time. The fluid remains grayish in color.

7. Tuberculous meningitis gives a pellicle formation which extends from the upper level to the bottom of the test tube. It appears in either a thread-like or cone-like formation, with small processes projecting sideward. The pellicle is snow-white in color (unless there is blood in it).

8. A pellicle forms in spite of serum injection.

9. A pellicle forms when the spinal fluid is mixed with blood.

10. A foam appears at the surface of fluids drawn from pathologic cases when the tube is but slightly shaken, and persists for some time.

11. A uniform method of taking spinal fluid facilitates all tests on spinal fluid.

THE "DELAYED NEGATIVE" WASSERMANN REACTION

BY GEORGE MANGHILL OLSON, M.D., MINNEAPOLIS, MINN.

THE Wassermann reaction is not infrequently negative when the patient shows signs of syphilis or very obviously has not had sufficient treatment. In late or tertiary syphilis the reaction may be negative when the patient has had no treatment at all. In order to increase the percentages of positives in syphilis, and to render the test more delicate, the Wassermann reaction has been modified by a large number of methods, and attempts at its replacement by other and entirely different tests have been made. The unfortunate result of this attempt to modify or cause the Wassermann test to give more positives in syphilis has been not only to cause more positives in syphilis, but to get positives in patients that were entirely free from syphilis. So that today the original Wassermann test is still the most useful, and is less liable to error than the many methods designed to replace it.

The "delayed negative" Wassermann does not modify the technic in any way, and is made simply by taking readings at twenty minute intervals while the tubes are in the incubator, instead of one reading at the end of two hours. The Wassermann test showing complete hemolysis at the end of two hours is read as negative. This hemolysis however often occurs in twenty to thirty minutes after the tubes have been placed in the incubator. The time at which this hemolysis occurs varies very markedly when testing different sera, and if only one reading is taken at the end of two hours, much valuable information will have been lost. Readings should be taken at intervals of twenty minutes, and if this is done, the "delayed negative" Wassermann will often be found. At the end of the first twenty minutes, often the negative control will be completely hemolyzed, there will be no hemolysis in the positive control, and the serum to be tested will show no hemolysis. In other words the serum to be tested shows a positive reaction at the end of the first twenty minutes. At the end of forty minutes, however, the serum to be tested shows marked hemolysis, while the positive control still shows absolutely no hemolysis. At the end of two hours the serum to be tested is completely hemolyzed or absolutely negative.

I have found this "delayed negative" Wassermann to be of very much greater value than the slightly positive Wassermann, where the test shows only some non-hemolysis at the end of two hours in the incubator. The slight positive Wassermann is often due to spoiled serum, errors in technic, etc. The "delayed negative" Wassermann is, I believe, of much greater value in showing the presence of some of the so-called antibodies of syphilis, even when they are not present in sufficient amount to give a positive Wassermann. In patients with the initial lesion of syphilis, this reaction is often present when the Wassermann reaction is absent. Any patient having a suspicious sore, and showing this "delayed negative" Wassermann, should be very carefully and repeatedly examined for the presence of the *Spirocheta pallida*. The reaction is also of importance in the tertiary stage, where patients with active lesions of syphilis show thirty-three per cent of negative Wassermann reactions. As a guide in the treatment of syphilis, the "delayed negative" is an indication for more treatment, even though the Wassermann is completely negative at the end of two hours in the incubator.

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EDITORIALS

The Etiology of the Common Cold

OF all the less serious diseases that afflict human beings, there is unquestionably none that leads to so much discomfort and that, economically speaking, reduces efficiency by a higher percentage than the so-called "cold in the head." There seems to be no lasting immunity and it goes through families, schools and institutions in a way that has long left no doubt as to its transmissibility from person to person. It has been investigated bacteriologically by a large number of workers, probably by many more than the literature shows. We alone know of two pieces of work done on this illness, which were never published because of their inconclusiveness. When the colds have been very severe, they have usually been called "grippe" or "influenza," in spite of the fact that the Pfeiffer bacillus was not often found. At various times, the influenza bacillus, the different streptococci and pneumococci have been held responsible. During recent years Tunncliffe described a bacillus to which she attributes etiological importance. It has been noticed by many bacteriologists, who have studied the condition, that the mucus from typical colds was curiously free from bacteria.

In 1914 Kruse¹ suggested that the ordinary cold might be due to a filterable

¹Kruse: *Munchener Mediz. Woch.*, 1914, vol. lxi.

virus. He filtered the secretions from the nose of a person suffering from a cold, through a filter impervious to bacteria, proved it free of bacteria and, with it, inoculated a series of volunteers, one-third of whom contracted colds within one to four days. In a second series of 36, he obtained positive results in 42 per cent.

Foster of the U. S. Army Medical Corps has recently repeated Kruse's experiment in the Laboratory of Preventive Medicine at Harvard. The case with which he began work was one of typical coryza with moderate systemic symptoms and profuse mucoid nasal discharge. From this and two similar cases, secretion was obtained, mixed with isotonic salt solution, and filtered through a Berkfeldt "N" filter. Bacterial cultures of this filtrate proved sterile. Ten volunteers from the garrison of Fort Banks were inoculated with the filtrate by instilling about three to six drops into each nostril, after washing the nose with water. Nine of these ten developed symptoms of acute coryza in from eight to thirty hours. Some of them developed simple rhinitis, others had slight fever, with involvement of the eustachian tubes, sore throat, cough, and pain in the back; one of them had tenderness of the parotid, another herpes of the lips. These cases of Foster seem definitely to confirm Kruse's observation and, in the light of these results, we may be permitted to add a single instance observed by Dr. Hopkins and the writer, which appears now to have more importance than we ventured to attribute to it at the time. A secretion, filtered and controlled just as were those of Foster, was obtained from the nasal mucous of a student at Columbia, suffering from acute coryza, and about 1 c.c. instilled into the nose of a member of the laboratory staff. He developed a violent coryza within three days. However, this individual had been exposed, as had the rest of the laboratory staff, to intimate contact with the class, a high percentage of whom were suffering from acute coryza at the time, and control experimentation could not, therefore, be carried out. This is mentioned only because of the fact that it seems to us of great interest in connection with Foster's excellent investigation.

Particular importance is lent to Foster's work by the fact that he went on to the cultivation of the apparently filterable virus by the methods employed by Flexner and Noguchi in the case of poliomyelitis, in which tissue ascitic fluid and tissue broth were used in high tubes sealed with paraffin oil. About 0.5 c.c. of the nasal filtrate was introduced into the tubes, directly in contact with the tissue, after these tubes had been proved sterile by previous incubation. A distinct, grayish-white, opalescent halo appeared at the end of twenty-four hours, in the ascitic fluid and broth. This thickened for three or four days and the haziness extended up the tubes. Under the dark field, active, minute bodies, occurring singly and in groups, appeared and, stained by various methods, minute coccoid bodies were demonstrable in all the preparations. Successful inoculations were obtained from eleven soldiers into whose noses the filtrates obtained from sub-cultures and so diluted that the original material obtained from the first case was in concentration not higher than 1,000 to 90,000.

We regard the work of Foster as of unusual importance. His results are clear-cut and, although in the original conception a confirmation of Kruse, go much further in that they may possibly have resulted in the successful cultivation of the microorganism. It is further worthy of comment that Foster's work,

though systematically and very accurately carried out, is presented with a degree of conservatism and self-criticism which lends additional weight to the conclusions which he himself regards as justified.

—H. Z.

Death by Suffocation

DEATH from suffocation may result in the following ways:

1. From the growth of a tumor in the larynx or trachea.
2. By disease of the glottis, such as edema, or by spasm produced by some powerful irritant, such as boiling water or some corrosive poison. These modes of death by suffocation may be natural or due to disease.
3. The accidental entrance of foreign bodies into the larynx. There are many cases, especially among children in which death has been caused by bits of food getting lodged in the windpipe during swallowing, or by vomited matter getting into the windpipe.

Mackenzie tells of a man who choked while attempting to swallow a large piece of meat. Death occurred in a short time, and postmortem examination showed that the piece of beef had lodged in the glottis, a part of it having passed through the rim. In another instance a drunken man was found dead, and examination showed a thin piece of potato skin covering the rim of the glottis so closely that it prevented the entrance of air. The man had eaten potatoes for dinner, and while thoroughly intoxicated vomited, and being unconscious he was unable to cough, and death resulted from this mechanical obstruction. In still another instance cited by Taylor a woman died during a violent fit of coughing. An autopsy showed a large tough piece of mucus lodged in and closing the rim of the glottis.

4. A foreign body may be forcibly introduced into the mouth and crowded down upon the glottis so as to cut off the air supply. The introduction of such bodies may be done without the purpose of injury. Thus, nurses sometimes gag their charges with little bags made of soft leather or cloth containing some sweet substance. In several instances death has resulted in this way. The child, unable to remove the gag, carries it back into its throat until respiration is interfered with, and death may be induced. In some instances this gag is only a rag sweetened with sugar.

5. By too closely covering the head. It is easy to kill an infant by suffocation either accidentally or intentionally. The writer has known of several instances in which children only a few days old have gone to sleep closely pressed to the mother's breast. The mother also has fallen asleep, the child's head gets under the bedclothes, or is in part covered by the body of its mother, and it dies without a struggle. This is known as death from smothering or overlaying. In the great majority of instances it is unintentional, but cases of intentional infanticide by this method have been reported. Any person may be killed by smothering, provided the difference in strength between the victim and the murderer is sufficient to allow the latter to accomplish his purpose. In order to render his victim more helpless the homicide may resort to the previous

administration of some drug. As a rule homicidal death by smothering is inflicted only upon infants or the aged and infirm.

6. By accidentally burying the body in grain, flour, ashes, or some other finely divided substance. A man may fall down a wheat elevator, escape all violence from the fall, but may die in a few minutes from suffocation. Deaths caused in this way are probably always accidental.

7. The face may be held in snow, mud, ashes, feathers, grain, leaves, or some similar substances until death from suffocation results.

8. The hand or a cloth may be forcibly held over the mouth or nostrils until death results. Frequently the homicide in resorting to this method aids in the accomplishment of his purpose by placing the weight of his body upon the chest of his victim.

It is quite evident from what has been said that even homicidal death by suffocation may result without any external evidences of injury. In investigating deaths from suffocation, the expert must carefully note the condition of the larynx, and especially look for the presence of foreign bodies in this location. Tardieu once believed that the presence of minute ecchymoses on the surface of the lungs beneath the pleura was characteristic of death by suffocation. He described these spots as varying in size from that of a pinhead to a lentil, and in number from five or six to so many that the lung had a granite-like appearance. They are found most abundantly near the base of the lungs. However, other investigators claim that these ecchymoses although common in death from suffocation, are met with in other forms of asphyxia, and a committee of the medico-legal society of Paris made the following report concerning them:

1. Subpleural ecchymoses may appear after death from various causes:
2. They may be met with after death from hanging, strangulation, submersion, and suffocation:
3. They are of value only when associated with other signs indicating the cause of death.

The condition of the heart is variable, but as a rule corresponds with that found after death from other forms of asphyxiation. The blood is fluid and the right heart distended, while ecchymoses are frequently found in the pericardium. The brain as a rule is engorged with venous blood, and there may be hemorrhages, although these are rarely found.

Suffocation is the cause of death in most persons killed by crowding. When people are seized with panic and rush from a building most of those who lose their lives are suffocated and not trampled to death. The body is so compressed by the crowd and the volume of air so limited, that death from suffocation results. This may happen, if the crowd be sufficiently dense, in the open air. Indeed, the most striking illustrations of this kind of death have occurred in the open, as for instance the death of more than 1,500 persons in the open plain near Moscow at the time of the coronation of the present Emperor of Russia.

Suffocation from Disease.—It should always be borne in mind that every sudden death from suffocation is not due to violence, either suicidal or homicidal, nor to accident; and whenever an obduction is held the man or men who perform it should see whether or not death may have been due to some patho-

logical condition. The presence of a tumor is so easily recognized that it needs only to be mentioned. Laryngeal diphtheria, fortunately much less common now than before the discovery and application of diphtheria antitoxin, is still an occasional cause of sudden death, although as a rule death is preceded by some days of illness. It is known that a small plug of bronchitic mucus may suffice in infants to close one of the small bronchi and cause sudden death. In some instances this obstruction to the passage of air is so small that it can be detected only by careful microscopic examination. Herford, as quoted by Ziemke, reports six cases of sudden death in children in which no macroscopic cause could be found, but histological examination proved the existence of capillary bronchitis. It seems that there are instances in which the thyroid enlarges without attracting attention and finally interferes with respiration and may cause sudden death by pressure on the blood vessels or by reflex action through the nerves. Richter thinks that in these instances there may be marked hyperemia and swelling in the tracheal mucous membrane. Again, there is the condition known as status lymphaticus in which there is general hyperplasia of the lymphatics, involving the thymus, the solitary and agminated glands in the mucous membrane of the intestines and the lymphatic glands in other portions of the body. It has been supposed by some that the hyperplastic thymus in children through either a very rapid enlargement or by pressure being brought to bear on some nerve by a sudden movement may sometimes cause sudden death. These explanations are largely hypothetical and not altogether satisfactory. Ziemke states that in some of these cases he has found catarrhal changes in the lungs and more frequently in the intestines. In the instance of a child one year of age that had repeatedly shown signs of heart weakness and had suffered from intestinal catarrh, he found hyperplasia of the thymus with ecchymoses, a marked status lymphaticus, an extraordinary dilatation of the left ventricle and a fatty degeneration of the cardiac muscle. He also states that in cases of sudden death in children in which evident changes are lacking, he has never failed to observe a well developed rachitis. In such instances there may be the condition designated by Kassowitz as "expiratory apnea" and spasm of the muscles of the glottis may account for the sudden death. The theory of a suddenly developed endogenous poison is of special interest in connection with the recent discovery of the fact that many proteins contain a poisonous group, but much work along this line must be done before positive statement can be made. The subject of a sudden death in early life with the literature has been admirably presented by Griffith.¹

Sury² gives an extensive review of the literature of so-called *mors thymica*, adds considerable of value from his own studies, and reaches conclusions that may be abbreviated as follows: None of the reports of cases of death from this cause in the newly born are free from error. Aspiration of fluid into the bronchi may happen in so-called easy births when the pains follow in rapid succession. The fetus suffers from oxygen hunger and breathes too soon. As a rule, the size of the thymus varies with that of the child, and in many instances in which the gland has been reported as "enormous" its weight falls within normal limits. Sudden death of a child from mechanical causes in case of normal thymus have not been demonstrated. There is in these cases a natural cause of death, and this is generally either a bronchitis or an enteritis, which

is overlooked at the obduction. Chronic difficulty of respiration, as continued stridor and partial asphyxiation, rarely, though sometimes, seen in children, is benefited by partial or total extirpation of the thymus. The explanation of this is not clear. In only one case, that of Rolleston, was the gland found to weigh more than 300 g. Complete closure of the trachea in cases of so-called enlarged thymus by throwing the head backwards, has not been proven. Dangerous pressure on the vessels or nerves by a thymus of normal size has not been demonstrated, and seems improbable. The so-called typical signs of asphyxiation are not pathognomonic, and consequently are not of value in forensic medicine. A positive diagnosis of status lymphaticus in a child is difficult, and should be made only after a most careful study. The involution of the thymus begins with adolescence, and there is a relation between this gland and the sexual organs not yet understood.

Suffocation in Attempting to Swallow.—When one attempts to swallow something that is too large to pass into the esophagus, it may get so wedged into the throat as to occlude the larynx. Unless the object is speedily dislodged, dyspnea, spasm and loss of consciousness followed by death may follow. The pieces that men have attempted to swallow are of surprising size. Richter found a piece of meat 8 cm. long, from 3 to 4 broad and 2 thick, and Liman found a whole herring rolled up and lodged in the throat. People go to sleep with things in their mouths and these may be aspirated into the air passages. Rubber balls and similar playthings given to children are dangerous and not infrequently cause death. Diehl tells of a man who while springing across a brook aspirated a cigar stump into his air passages and was dead within a few minutes. Articles of food vomited from the stomach may be aspirated into the lungs with fatal results. Accidents of this kind are most likely to happen to children and to drunken adults. When the aspirated particles are small they may be carried into the finest bronchi. Severe hemorrhage or the discharge of an abscess may fill the larynx and cause death by asphyxiation. Cases are reported in which the trachea has been occluded with necrotic tissue from caseous lymph glands that have perforated into the bronchi. In death from accidents of this kind the lungs are found to be inflated.

Closure of the Nose and Mouth.—When this is done with the naked hand the force used generally exceeds that necessary and scratches with the nails are frequently left in evidence. When the hand is covered or some soft material is laid over the face and pressure made upon it the marks will, of course, be wanting. Children are often killed in this way or they may be smothered by being covered or by being buried in ashes, loose dirt or in meal or flour. When finely divided material is employed particles may be aspirated into the lungs. Infants and adults in an unconscious state may be suffocated by lying face downward in any soft material. It is said by Okamoto, that a few thicknesses of moist Japanese paper placed over the nose and mouth of an infant excludes the air sufficiently to lead to death in a few minutes. Suicide by closing the mouth and nose with the hand is unknown and is probably impossible. Ziemke states that there is recorded one instance of suicide by putting the head under a mass of bed clothing. A woman killed her child and herself in this way. Taylor states that formerly in London a thief would knock his victim senseless and while in this state cover his nose and mouth with a pitch plaster.

Suffocation by Pressure on the Chest.—If external pressure on the chest be continued for a short time and be sufficient to markedly retard the respiration death occurs. This is not an uncommon cause of accidental death. For instance, men are digging a trench in a gravel soil and have reached a depth of four to five feet when a mass of earth falls about them. Unless speedily extricated death may result from the external pressure. Indeed, the depth of burial may include no more than the abdomen, and if the pressure be continued it will arrest the movements of the diaphragm and cause death from asphyxia. When people are jammed together or against a wall in a mad rush in the face of some threatening catastrophe some become unconscious and fall on account of the pressure. After falling they are likely to be trampled upon and the injuries resulting from this are generally reckoned as the efficient causes of death. It is easily understood that the mechanism of death by compression is quite different from that induced by any other form of strangulation or suffocation. There is increased blood pressure in every part of the body and ecchymoses are numerous. When the head as well as the trunk is buried two factors may come into play, the partial exclusion of air from the upper passages and the relative immovability of the respiratory muscles. When one is rescued in time to save life the subsequent symptoms will vary with the degree of the pressure and the time it has been borne, but there may be protrusion of the eye balls, fixed pupils, generally in dilatation, possibly complete blindness, and albuminuria. Paralytic symptoms may also be present and psychical disturbances are sometimes observed.

In cases of death the skin has an ashy or bluish-black appearance and on close examination it will be found that this color is not wholly due to cyanosis but that there are numberless ecchymoses.

Death by compression of the thorax is in the great majority of instances accidental. The pressure of the knee on the chest in some cases of homicidal strangulation may be a contributory factor in the causation of death, and Maschka reports a case in which a girl was raped by two men. While one attempted to perform the act the other held her by pressure on her thorax and this killed her.

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—V. C. V.

Acidosis

PERHAPS if the present tendency persists in the elucidation of disease processes we will say that streptococci (or pneumococci), acidosis, and ductless gland disorders cause all diseases. Whether such a trend does or does not persist, there is evidence that there is a great deal of conversational and literary excitement centering about acidosis. Any serious logical discussion of that topic is therefore welcomed, and such is that of Howland and Marriott.¹ Their discussion has particular reference to children, but is not however narrow be-

cause of that reference. They call attention to the fact that acidosis is very loosely applied in medicine and that, as a rule, it is taken to mean that acetone bodies have been found, by qualitative tests, in the urine. They also say that acetone in the urine is not necessarily abnormal. In other words it is not the mere presence of acetone bodies that determines acidosis; acidosis depends upon the relation between acid production and neutralization in the body. The presence of acid in the urine may not be the expression of an acidosis, for a severe acidosis may exist with no abnormal amounts of urinary acid.

There are several ways of discovering the presence of an acidosis. One of these is to discover the ammonia excretion. A high ammonia coefficient always arouses the suspicion of acidosis but unless there is other confirmation, is not conclusive, for it is to be remembered that a high ammonia excretion may result from dietetic causes. Fatal acidosis may occur without any considerable increase of the ammonia coefficient.

Another method is to determine the carbon dioxide tension of the blood. It is diminished in acidosis. It is diminished because there is less alkali (bicarbonate) in the blood. Sodium bicarbonate is the great neutralizer of the body. If there is a relative excess of carbon dioxide over bicarbonate the neutral reaction of the blood moves to the acid side, the respiratory center is stimulated, and by increased pulmonary action, the body attempts to remove the excess of CO_2 . So, bicarbonate deficiency of the blood is an indication of acidosis, as is also, dyspnoea. The carbonate content may be tested by Sellards' method² applied to the serum, or by the alkali tolerance test.^{2, 3} The former consists in removing the proteins of blood serum with absolute alcohol and evaporating the filtrate with a few drops of phenolphthalein. Normally a deep purple color appears. According to the degree of carbonate deficiency the color is paler, or absent. The latter test depends upon giving sodium bicarbonate by mouth until the urine is alkaline.

Howland and Marriott have used these methods and give details of cases in which they were applied. They also remark that in absence of proof of presence of acids or loss of bases, a striking evidence of acidosis in children is decrease of urinary secretion.

They make the following statement, "We may lay it down as a general maxim that as hyperpnea indicates acidosis, so hyperpnea indicates alkali therapy, and this for infants or older children." They proceed to say that alkali may be given by any route, but in case rapidity of action is necessary, it is best given intravenously. They mention the method of using the superior longitudinal sinus, or the external jugular or femoral veins. They use only the bicarbonate, —4 per cent for intravenous use; 2 per cent for subcutaneous,—and advise its continuance until the urine is alkaline.

The remarkable things about this contribution are its brevity and lucidity, and the absence of any reference to Fischer's work.

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Recent Studies on Hemophilia

THERE are few fields in internal medicine which are more obscure than that of the so-called hemorrhagic diseases. The purpuras form a group of poorly differentiated conditions, oftentimes overlapping in their clinical manifestations. Not until we have more exact knowledge of the hemotologic changes will it be possible to classify purpuras satisfactorily or to apply rational treatment.

Hemophilia is one of the hemorrhagic diseases. It has been recognized clinically since 1803, when it was first described by Otto of Philadelphia. He recognized the hereditary nature of the disease and pointed to the fact that it is transmitted by the female, who is herself unaffected by the disease, but may transmit it to her male offspring.

The cause of the tendency to prolonged hemorrhage in hemophilia has been sought by numerous observers. The striking fact has been noted by many that when blood is removed from the hemophilic unmixed with tissue juices, its coagulation time is greatly delayed, often to two or three hours or more. The reason for the delay has been variously given.

According to Howell's theory of coagulation of the blood, there are five essential factors, i.e., prothrombin, antithrombin, thromboplastin, fibrinogen and calcium. With the exception of thromboplastin, which is present in the tissue-juice, all of these substances occur in the circulating blood. Intravascular clotting is prevented by the interaction of the prothrombin and antithrombin. When, however, thromboplastin is added to the blood, it combines with the antithrombin, and thus the prothrombin is set free. The prothrombin is now converted, by the action of calcium, to thrombin. This, in turn, changes the fibrinogen into fibrin, a clot resulting.

With this theory in mind, Addis¹ approached the problem of hemophilia and presented evidence which indicated a qualitative defect in the prothrombin of the blood.

Howell,² in 1914, reported his own studies on the blood in hemophilia and other conditions. He devised methods for determining the approximate amounts of antithrombin and of prothrombin in the blood, and applied these methods to the study of three cases of hemophilia, whose spontaneous clotting times varied between two and one-half and five hours, when blood was obtained unmixed with tissue-juice. The importance of careful technic in obtaining the blood was well illustrated by one of his cases. On one occasion the needle missed the vein and only after several trials was it possible to obtain 2 c.c. of blood. When emptied into a tube, this blood clotted in ten minutes, a normal time. With perfect technic, a second specimen was obtained from a vein of the other arm; it required between four and five hours to clot. It is evident, therefore, that admixture of thromboplastin from the tissues greatly lessens the clotting time.

Examination of these bloods for antithrombin content showed a normal amount of this substance, or possibly a slight increase at times, though Howell began his studies with the expectation that a constant excess of antithrombin would be encountered as the cause of the delayed clotting.

The prothrombin content of the blood was also studied. In all the specimens examined from the three patients at various times, there was found a deficiency in the quantity of prothrombin, as compared with normal blood.

Howell, therefore, says of the disease, "Hemophilia may be defined as a condition, limited to the male, in which the coagulation time of the blood is markedly prolonged in consequence of a deficiency in the amount of the contained prothrombin, with the additional characteristic that the defect is transmissible by heredity in accordance with the so-called loss of Nasse." He further says, "The ultimate cause of this condition cannot be stated. So far as we know, the prothrombin present in blood plasma is furnished by the blood platelets, and it is reasonable to assume that the defect in question is referable to some functional change in these elements."

The conclusions of Howell regarding the deficiency in the quantity of prothrombin in the blood in hemophilia have been confirmed by the recent work of Hurwitz and Lucas,³ who have studied five cases of hemophilia, in all of whom prothrombin deficiency was found, with normal quantities of antithrombin.

By the local application of kephalin, a special phosphatid isolated by Howell (thromboplastic substance), they were able to cause an early arrest of hemorrhage in hemophilics.

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—R. S. M.

The Pituitary and Growth

FROM very early times, even those of Galen and Vesalius, it has been suspected that the pituitary had a secretory function, though some intermediate writers believed that this function had only to do with the production of cerebrospinal fluid. Liegevis (1860) was the first to place this gland with the organs of internal secretion—with the ductless glands. But it was not until 1886 that Marie offered evidence that this organ had a sure effect upon the metabolism of the body and that it had an intrinsic importance in the growth processes. Even now, after many years of experimentation and consideration, the mechanism underlying the change in metabolism is not clear. The factor of age is one which must be considered, however, for young animals and adult animals behave differently in their reaction to experimental procedures. If in puppies a part of the gland is removed they become fat, slow, and remain undeveloped physically, and fail to acquire normal secondary sex characteristics. If adult animals are subjected to the same procedure, they lose some, at least, of their acquired sex characteristics and become less fat and less inactive than the younger animals.

During hibernation, the pituitary undergoes a sort of physiologic atrophy and this process is associated with increase of fat, lethargy, low temperature, and general physical inactivity, symptoms which are similar to those resulting from experimental damage to the gland. As the period of hibernation passes, the pituitary becomes normal in appearance and the symptoms are lost. Feeding gland substance after destruction of the gland produces similar speeding up of metabolism.

Not a few experiments upon the effects of feeding pituitary substance to, or injecting pituitary extracts into, animals have been made—with very contradictory results. Crowe, Cushing and Homans,¹ for instance, found that repeated subcutaneous injections of sterile extracts or emulsions of the whole gland, or of posterior lobe alone, given subcutaneously, were apt to lead to emaciation. Caselli, on the other hand, was able to observe no appreciable effect upon growth after long continued injections of glycerine extracts of the whole gland. But Cushing nevertheless believed that growth was retarded by feeding pituitary material. Sandri reported negative results after feeding young mice with bovine anterior lobe, but feeding with posterior lobe arrested development. Schaefer on the other hand, observed a definite increase in growth of young rats fed upon anterior lobe extracts. Aldrich and Lewis and Miller² could perceive no obvious effects produced by feeding pituitary substance to young rats. Wulsen³ using fresh anterior lobe of ox pituitary observed retardation of growth. Behrenroth is, Goetsch says, the only author who makes special mention of an accompanying effect upon another of the ductless glands. He reported that pituitrin given at proper intervals produced premature development of the sexual organs and coincident increased sexual activity on the part of the animals (young rats).

It was because of the contradictory results of pituitary treatment as reported in the literature, and because of the quite obvious correlation of the sexual glands with the pituitary, that Goetsch has carried out a series of experiments⁴ with young rats, of known pedigree, kept under the best conditions, fed with pituitary substances in the form of powdered extracts of the various parts of the gland. His reported results are as follows:

Dried powdered pituitary extract derived from both anterior and posterior lobes, when fed to young rats in excessive doses (0.1 gm. daily), causes failure to gain in weight, loss of appetite, increased peristalsis, a mild enteritis, and certain nervous manifestations, such as muscular tremors and weakness of the hind limbs. The latter symptoms seem to be due to posterior lobe elements in the extract. When whole gland is fed over a short period of time, not in excessive doses, it causes a more rapid growth and development, gain in weight, larger nipples in the female, and a coarser, drier, harsher growth of hair than are seen in either control animals or after similar administration of ovarian extract in equivalent dosage. Whole gland extracts also influence the female sex glands, increasing their development and activity. In males there is an analogous stimulation. In other words, sexual precocity results in either sex.

Feeding with anterior lobe extracts produces the same effects as feeding whole gland, and also exerts a stimulant effect upon the fetuses *in utero*. Extracts of posterior lobe on the contrary seem to have a retarding influence upon the sex glands, and one which is quite similar to the effect of ovarian extract on the testes. Moreover posterior lobe extracts do not stimulate growth in general. Large doses cause loss of weight.

Ovarian extracts have stimulating effects upon the female and retarding influence upon the male sexual development.

These results explain, as Goetsch says, the fact that in acromegaly which is due to overfunction of the anterior lobe, the early stages are characterized by

increased sexual activity and in the late stages loss of sexual function. In the later stages the pituitary is exhausted or destroyed.

The chief value of the work seems to be that it offers indications that pituitary therapy may be successful. Goetsch points to the fact that pituitary feeding in loss of sexual activity due to hypophysis disease has resulted in a return, partial or complete, of those activities.⁵ He also suggests supplementary feeding after surgical operations upon the pituitary, and also in conditions characterized by genital aplasia, adiposity, and under-development, pituitary feeding may be valuable, with extracts of other glands when necessary. Also it is possible to treat certain conditions of sexual overactivity with posterior lobe extracts, or, in males, with corpus luteum extracts.

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—P. G. W.

Splenectomy

IN a recent number of this Journal,¹ attention was called to the relation between the liver and spleen, and to the function of the spleen. In the remarks made at that time it was stated that "heretofore splenectomy has been an experiment. It has been done as a last resort. But if in certain anemic conditions it can be shown that abnormal hemolysis is fundamental, and that the anemia is due to it rather than to a lack of production of blood cells or pigment, then it will be logical and not merely empirical to remove the organ which furnishes the normal augmenter of hemolysis." At the same time it may be that experimental removal of the spleen may show whether or not the spleen is overfunctioning in producing the augmenting substance which activates, so to speak, the normal hemolytic substances of the liver.

In a recent article, W. J. Mayo² recounts the results he has had with splenectomy. He divides the cases into four groups; those in which the spleen is the seat of a primary tumor; those in which the spleen is the seat of an infectious process; those in which it shows enlargement associated with disease of the liver; and those which are associated with the so-called blood diseases. The first group he leaves out of account.

Among cases belonging to the second group, he places one of, apparently, primary tuberculosis. Temporary improvement was followed by death after several months from general miliary tuberculosis. Also belonging in this group, were two cases of chronic syphilis with splenomegaly and anemia. After removal of the spleen there was a "marvelous" improvement of the anemia. In other conditions such as chronic recurrent sepsis, the spleen has been removed in seven instances. The outcome is not stated. The context indicates that it was not good.

The results in the third group have been not much better. In one case of

Hanot's cirrhosis, splenectomy gave undoubted benefit. In four instances of portal cirrhosis, splenectomy has been performed with excellent temporary results—disappearance of the ascites and anemia. If, as is suggested, the noxae which cause cirrhosis come from the intestinal tract then there seems to be no good reason for making the experiment, unless the experiment is justified by a purely temporary relief. We believe such an experiment is justified, but are unable from the data to expect a cure.

In the last group the results of splenectomy are truly brilliant. In splenic anemia, Banti's disease, and Gaucher's disease, cases were definitely improved, and, if operated upon early in the disease, may be cured. Most interesting perhaps in this group is hemolytic jaundice. In this group splenectomy has been done in nine cases with striking results. "Within twenty-four hours the jaundice begins to disappear and in a few days the patients, perhaps for the first time, have clear complexions; the anemia is rapidly overcome and they remain well." In pernicious anemia also, after an experience with 19 patients, the results seemed promising in selected and early cases.

These surgical experiences bear out the conclusions which are drawn from experimental work. The brilliant results appear in cases in which hemolysis is the salient feature, and in such cases it is the removal of the source of hemolysis augmenting substance which is productive of good. The results must also always be good in cases of primary diseases of the spleen which tend to spread therefrom, or which will tend to render the organ useless. It is possible that in some cases of pernicious anemia, part of the fault lies in splenic overactivity; in such cases splenectomy should be valuable. In other cases, those of purely myeloid origin, it would seem to be useless to remove this organ. In any case of anemia, a temporary improvement might be expected, if it be true that the spleen has a part to play in normal blood destruction.

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—P. G. W.

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ORIGINAL ARTICLES

THE SIMPLE INTERPRETATION OF POLYGRAPHIC TRACINGS*

BY EDWARD PERKINS CARTER, M.D., CLEVELAND, OHIO.

IT is to Mackenzie¹ that we are indebted for having applied the fundamental principles of the physiology of the circulation to the study of clinical cases, and for having devised, amidst the exigencies of an absorbing practice, the most satisfactory means for simultaneously recording the venous and arterial tracings.

The soundness of the graphic records in their application to cardiac pathology has been abundantly confirmed by animal experimentation and by the electro-cardiograph as originally developed by Einthoven and so brilliantly applied by Lewis.

FUNDAMENTAL PRINCIPLES.

The basis of the analysis of all polygraphic curves depends upon the *time relationship* between the events recorded in the venous and arterial tracings. Their accuracy, to within an utterly negligible factor of error, lies not in any given form or contour of the various waves upon the tracing, but solely upon the orderly or disorderly sequence of events as shown by an exact analysis by measurement from fixed points.

The venous pulse in the jugular vein is due entirely to changes in pressure occurring within the right auricle, and in order to interpret correctly our venous tracings one must have a clear understanding of the variations in pressure occurring within the auricle during the cardiac cycle.

As a result of experimental work with animals we know that graphic records obtained by sounds introduced through the veins into the right auricle always show first a wave directed upward, due to an increase in pressure within the auricle during auricular systole—the first positive wave. This wave ends shortly before the onset of ventricular systole and is followed by a slight drop in the tracing called the first negative wave. This depression is immediately followed by

*From the Medical Clinic of the Cleveland City Hospital and the Western Reserve University.

a second wave in an upward direction coincident with the onset of ventricular systole—the second positive wave, which in turn is followed by a conspicuous fall in the tracing—the second negative wave. Following this second fall there occurs a third wave in an upward direction, the sharp onset of which appears at a variable time after the second negative wave, the termination of this wave, however, occurs at a constant time interval after the second positive wave, its apex coinciding with the opening of the auriculo-ventricular valves which takes place but a fraction of a second after the closure of the aortic valves. This third wave in an upward direction is called the third positive wave.

Lewis² states that “its dependence upon a ventricular rather than upon an auricular event has been clearly established experimentally and clinically.” * * * “That the rise of pressure in the auricle due to ventricular systole is a factor of great importance.” * * * “That it has been associated also with the sudden release of the base of the ventricle at the commencement of ventricular diastole.” * * * “It is probable that both factors play a part in the production of the wave under certain circumstances.”

The fact, however, that its production is essentially due to the rise in venous pressure in the right auricle as this chamber fills during diastole, the end of its rise terminating abruptly with the opening of the auriculo-ventricular valves, makes it difficult to interpret this wave as *positive* in the sense of that term as applied to the first two positive waves.

Following this third wave in an upward direction there succeeds the third negative wave coincident with ventricular diastole.

METHOD OF PROCEDURE.

To obtain satisfactory venous tracings from clinical cases the jugular tambour of the Mackenzie ink polygraph, or a small thistle funnel in its stead, should be placed over the jugular bulb, on the right side of the neck, at a point a little above and about an inch to the right of the sterno-clavicular joint. When possible the patient is placed in a horizontal position, the head being slightly elevated and the muscles of the neck relaxed. When it is not possible to place the individual flat one must adapt his procedure to the exigencies of the case. We may place the tambour at a higher level, or may often succeed in getting satisfactory tracings from the left side when only failure results from adopting the customary right side. The radial tambour should be connected to that pen or writing lever nearest the patient.

In a normal jugular tracing a regularly recurring series of waves is recorded grouped together in sequences of three more or less conspicuous summits with a fall in the tracing between them, a slightly longer pause occurring between the third wave of each group and the first wave of the following group. (See Fig. 2,A.) These three waves have been named “a,” “c” and “v,” these letters indicating the origin of the waves.

- “a” The first positive wave.
- “x” The first negative wave.
- “c” The second positive wave.
- “x” The second negative wave.
- “v” The third positive wave.
- “y” The third negative wave.

The "a" wave is due to auricular systole. Under certain conditions, referred to below, the "a" wave may be absent from the venous tracing.

The "a" wave and the following depression, measured from the onset of "a" to the onset of the "c" wave have a duration of from .1 to .2 seconds. This constitutes the so-called "a-c" interval, and represents the time which elapses between the onset of auricular and ventricular systole.

Whatever may be the exact production of the "c" wave its dependence upon ventricular systole has been established, and the occurrence of the "c" wave in the venous tracing synchronous with the appearance of the carotid pulse in the neck makes it the most important wave in our analysis and gives us a fixed standard upon which the correct interpretation of all tracings depends. Any increase in the "a-c" interval over .2 seconds is regarded as pathological, constituting delayed conduction, or a slight degree of heart-block.

The onset of the "v" wave varies under different conditions, its termination however corresponds exactly with the opening of the tricuspid valves and occurs at an interval of approximately .4 seconds after the onset of the "c" wave. It not infrequently happens that the "v" wave is split, a fact considered by Lewis as affording evidence of the double factors in its production.

It is upon the recognition and time relationship of these three waves as determined by comparative measurements upon the arterial tracing that the accuracy of an analysis of the venous curve depends.

THE ARTERIAL SPHYGMOGRAM.

In any analysis of an irregularity of the heart we must determine whether or not there is present a *dominant rhythm*. By this term is meant that fundamental physiological rhythm which arising at the sino-auricular node controls the heart beat. Under normal conditions this dominant rhythm originates the cardiac response at a uniform rate of 72-76 to the minute. It should be recalled, however, that the normal impulse arising at the sino-auricular node is subject to wide variations in rate, ranging from 32 to 100 or above a minute.

This dominant rhythm may be modified in various ways, as follows:

1. Physiological variations caused by an increase in the rate of stimulus production at the pacemaker.

- a. These are met with essentially as an increase in rate due to stimulation of the sympathetic, or to factors interfering with vagus inhibition.

- b. Slowing of the normal rate from vagus stimulation or irritation.

- c. Certain so-called phasic variations attributed to the action of the vagus; seen conspicuously in children as changes in rate with in- and ex-piration.

2. Pathological variations caused by,

- a. Abnormal impulses arising elsewhere than at the sino-auricular node, called ectopic.

- b. By interference with, or complete interruption of the passage of the normal impulse from auricle to ventricle.

We establish the presence of a dominant rhythm by the regularly recurring incidence of beats at points which are equal distances apart, or by demonstrating that the apparently irregular longer spaces appearing in a tracing are but simple multiples of the shorter beats which represent the dominant rhythm. This is spoken of as the "spacing" of the arterial curve. When there is no evidence of

a dominant rhythm present in a tracing, when that is the beats are all of varying lengths without any periodic slowing or quickening of the pulse rate, when no two phases of irregularity are identical in duration and it cannot be proven that the long intervals are simple multiples of the short beats, then the pulse is said to be totally irregular.

THE ANALYSIS OF THE VENOUS CURVE.

We determine first that wave in the jugular tracing occurring synchronously with the carotid pulse. We know that the radial wave at the wrist occurs .1 of a second after the carotid wave in the neck, and this fact gives us the means by which we can determine the "c" wave in the venous tracing.

With the jugular and radial tambours in place run off a short strip of venous tracing, then without moving the jugular tambour, stop the polygraph making an ordinate upon the paper by moving the pens quickly from side to side—these ordinates are called index marks; now without having shifted the position of the jugular tambour apply firmer pressure and secure a short strip of carotid tracing, making similar index marks upon stopping the instrument. We now have two tracings, venous and carotid together with the radial, upon which we can determine accurately by measurement the time relationship of the events occurring in

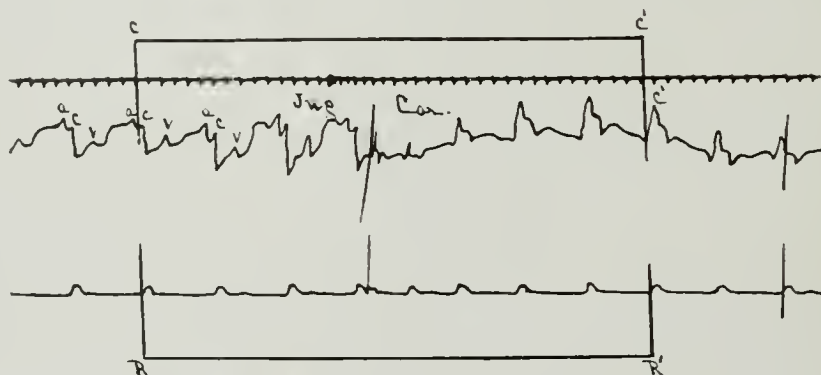


Fig. 1.—To illustrate the method of determining the "c" wave in the jugular tracing. Both jugular and carotid tracing taken without moving the jugular tambour.

the jugular and carotid tracing to the up-stroke of the corresponding radial. All measurements being made from the index marks at the right on to the tracing at the left.

We establish first the carotid up-stroke C' corresponding to a given radial wave at the right of the tracing; we then measure off an equivalent number of beats on the radial tracing on the venous side at the left, we may call this distance $R-R'$.

If we now transfer this measurement $R-R'$ to the upper tracing placing the point corresponding to R' at C' , a vertical line from R will fall upon a wave in the jugular tracing which bears the same relationship to the up-stroke of its corresponding radial wave R as does C' to R' , this wave therefore is the "c" wave and we may mark it as such. This procedure can be applied only when the polygraph is running at the same rate in both venous and carotid tracings, and we must be sure that the instrument when started after being stopped has developed the top speed at which it is set. The time marker in all tracings records fifths ($1/5$) of a second.

In routine work this method of procedure is unnecessary. We simply meas-

ure the distance from the index mark at the right to the up-stroke of any given radial wave. Transfer this measurement to the time-marker tracing, lengthen it by one-half of the distance between any two points, i.e., by one-tenth ($1/10$) of a second, then transferring this measurement to the jugular tracing place one end upon the index mark and the other will fall upon the up-stroke of the "c" wave. In this way determine three or more "c" waves.

THE FIXATION OF THE "A" WAVE.

We next determine the "a" wave by locating that wave which recurs at a constant interval immediately before "c." In the normal jugular curve the "a" wave is found at a period slightly less or just equal to the distance between any two points on the time marker, i.e., at a point slightly under or exactly equivalent to one-fifth ($1/5$) of a second before the onset of "c." In a given tracing in which the "a" waves recur with normal regularity in their time relationship to "c" they will be seen to have essentially the same form from one cardiac cycle to the next, in some instances altered slightly with recurring regularity by the respiratory rhythm.

It is important to note the contour of the "a" waves in any given instance, for under some conditions it is by the comparison of such regularly recurring waves which space exactly that we are able to assign the origin of the wave in question to auricular systole. This is of the utmost importance in those instances in which there are additional "a" waves present having no relation to any corresponding "c" wave.

While this statement holds true for any large number of observations, there are, of course, many occasions when the "a" wave coinciding with a "c" or a "v" wave is greatly obscured and its location in the tracing must be determined by careful spacing from the established "a" waves present.

THE FIXATION OF THE "V" WAVE.

In the normal jugular tracing the "v" wave usually appears as a conspicuous peak at a constant interval after "c." For this reason it is an important landmark in the jugular curve and by virtue of its time relation to the preceding "c" is of great value in the analysis of all tracings.

As stated above the onset of the "v" wave is variable, its apex on the other hand coincides with the opening of the tricuspid valves and will be found to occur at approximately .3 of a second after the apex of "c." Having determined the "c" wave it is usually not difficult to fix the apex of the succeeding "v" wave in its relation to the preceding "c" and to the dicrotic notch in the radial. When the "v" wave is split appearing as two small waves or as a single wave with a bifurcated peak one must proceed cautiously eliminating any possibility of confusion from a supernumerary "a" wave, and must further show that the "c" wave and the split wave immediately following fall within the sphygmie period of the arterial tracing. The sphygmie period is the period of ventricular systole. If we measure the distance in the venous tracing from the onset of the "c" wave to the beginning of the drop in "v" (lines 3 to 5, Fig. 2.A) and transfer this measurement to the radial tracing, making an allowance of one-tenth ($1/10$) of a second for the difference in time between the carotid and the radial, we shall

find that our measurement will fall one-tenth of a second before the up-stroke of the radial and at the base of the dicrotic notch.

It often happens that the "v" wave does not appear as a conspicuous peak, being frequently masked by the succeeding "a" wave. Any increase in the speed of the heart is accomplished at a sacrifice of the diastolic period, hence not infrequently when the heart's rate is enhanced "v" and "a" may be fused.

THE VENTRICULAR VENOUS PULSE.

Under certain conditions no "a" waves can be found in the jugular tracing while the heart's action may be regular or irregular. This type of venous curve has been called the ventricular form of venous pulse. When the pulse is regular the absence of "a" waves from the venous curve may be due to:

1. Overfilling and distension of the right auricle, following greatly increased pressure on the right side of the heart.

2. When the cardiac rate is increased and the "a" wave falls back upon the preceding "v."

3. Rarely in the presence of a slow pulse rate (36-42) due to the existence of complete articulo-ventricular dissociation (complete heart-block) associated with auricular fibrillation.

When the pulse is irregular the absence of "a" waves from the venous tracing signifies *auricular fibrillation*.

This form of venous pulse is most commonly seen as a result of the latter condition. In addition to the absence of all "a" waves it is further characterized by the fact that all the conspicuous waves in the tracing lie within the sphygmic period. The general shape of the waves in the ventricular venous pulse tracing may vary widely from case to case, but no matter how great this variation in form the conspicuous summits will be found to occur during systole. The so-called plateau type is perhaps the commonest form met with. (See Fig. 13.B.)

OTHER WAVES OCCURRING UPON THE VENOUS CURVE.

Under certain conditions additional waves are met with in the jugular tracing. These extra jugular waves will be found during the long diastolic pauses when the heart's rate is slow. The origin of these may be attributed to three sources:

1. The second onflow wave appearing as a gradual rise in the venous curve during diastole. Due to distension of the veins when the heart is already filled, or to the gradual increase in pressure in the veins and heart during the venous filling.

2. A small wave appearing in mid diastole having a definite time relationship to the preceding "v," seen conspicuously with a very slow heart rate. This is called the "G" or the "H" wave, having been described independently by Gibson³ and Hirschfelder⁴ and attributed by these observers to "the snapping together of the auriculo-ventricular cusps at the end of ventricular filling."

3. In certain instances of auricular fibrillation when the heart's rate is slow irregular undulations due to the rapid and imperfect partial contractions of the auricle may be seen. These are called fibrillation or "f" waves.

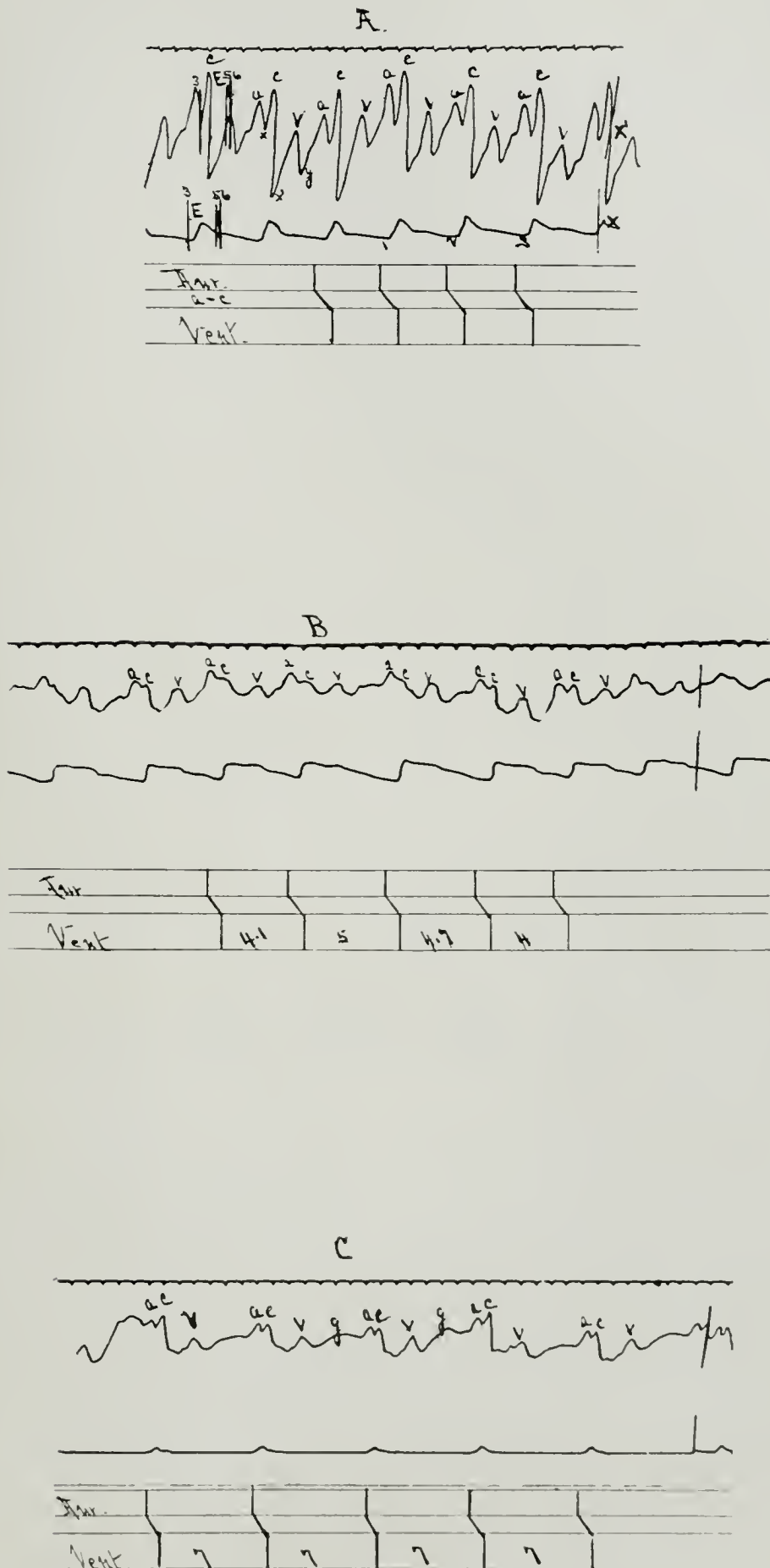


Fig. 2.—A. Normal jugular tracing. 3 to 5 sphygmie period. At 6 the tricuspid valves open. "a," "c," "v" and "x," "x'" and "y" the positive and negative waves.
 B. Sinus arrhythmia.
 C. Sinus bradycardia. Pulse rate 52 to the minute.

SUMMATION OF WAVES.

Whenever any two waves in the jugular curve coincide it is spoken of as a "summation." Not infrequently we see this summation of waves when the heart's action is perfectly regular though the rate is increased. In the sense of the term however in which it is commonly used it is applicable essentially to those instances of summation resulting from some disturbance of the normal mechanism.

We may find an "a" wave superimposed upon a "c" or upon a "v" wave; or a "c" wave upon "v" or "a." When this summation occurs the combination of "a" and "c" is usually more conspicuous than is that of "a" and "v" or "c" and "v." There are of course exceptions to this statement.

THE SIGNIFICANCE OF DELAYED CONDUCTION.

It is utterly unessential to the purpose in view to discuss in any detail our knowledge of the origin of the contraction impulse and its spread from auricle to ventricle.

We know that in the presence of a delay in the conduction time there oc-

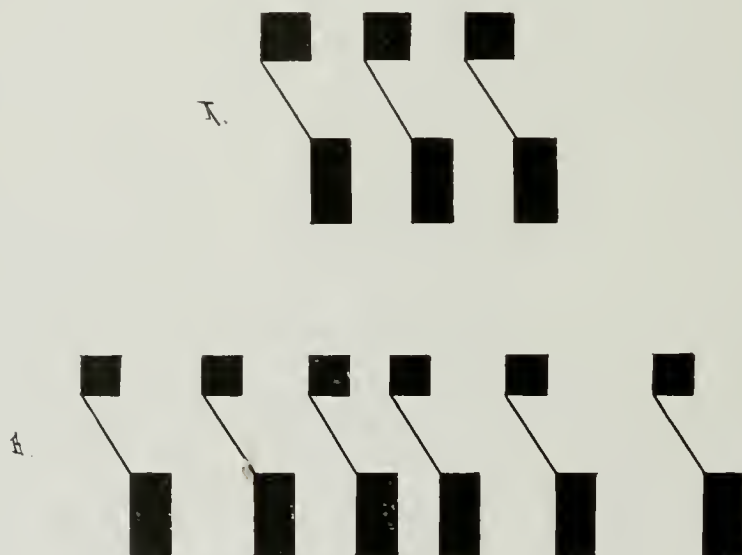


Fig. 3.—A. Diagram illustrating the normal mechanism.

B. Diagram illustrating a sinus arrhythmia.

In this and in all similar diagrams the upper squares represent auricular systole, the lower rectangles ventricular systole and the line uniting the two the conduction time, or a-c interval.

curs a change in the time relationship of the events taking place in the heart graphically portrayed in the jugular curve as a lengthening of slight or greater degree in the "a-c" interval. This constitutes the first degree of heart-block.

In the presence of the failure of the auriculo-ventricular bundle to convey the normal impulse from the pacemaker we find an isolated "a" wave, or with any of the higher degrees of heart-block alternately isolated "a" waves while in complete heart-block we can space the regularly recurring "a" waves and show that they have no constant time relationship to the "c" waves.

Depending upon the extent of impairment of conduction in a given instance there may be a considerable variation in the "a-c" interval present in a given case. This is particularly true of the higher degrees of heart-block in which we often find a rhythm of ventricular response varying between a 2:1, 3:1 and 4:1 ratio. In the simpler states in which there is present an occasional failure of conduc-

ion, spoken of as a dropped beat the first delay in the "a-c" interval exceeds the normal conduction time by a slightly longer duration than the second prolonged interval—occurring just before the dropped beat—exceeds the first. Immediately following the dropped beat the conduction time is again shortened.

Following the classification of Lewis⁵ we may consider in detail the simple interpretation of the common arrhythmias of the heart.

If in Fig. 2,A we measure the distance from the index mark "x" on the right to the up-stroke of the radial marked "l" add one-tenth of a second to this measurement and transfer it to the jugular tracing placing one end on the index mark "x'," the other end will fall upon the up-stroke of "c." Repeating this for "2" and "3," we then proceed to fix the "a" and the "v" waves, showing that they space equally and occur at a constant time interval in relation to "c;" "a" at slightly less than .2 sec. before, and the apex of "v" at .4 sec. after the onset of "c." The fall in pressure in the "v" wave will be found to occur at approximately .3 sec. after the fall in "c." This measurement may be used in obscure curves.

The further method of analysis is shown beneath the tracing, the upper row of vertical lines representing auricular, the lower ventricular systole, the line joining the two indicates the conduction time or "a-c" interval. These vertical lines are drawn at points corresponding to the onset of "a" and "c." It must be remembered that as the pens write in an arc of a circle all measurements must be made from the index mark at the right on to a line parallel with it at any given point and when possible at the same level.

The three negative waves are marked x, x' and y. The period E between the lines 3 to 5 corresponds to the sphygmie period. The measurements in the analysis beneath the tracing represent fifths of a second and fractions thereof, easily obtained from the time marker tracing. The diagram below illustrates the conventional method used to portray the sequence of events taking place in the cardiac cycle. The upper small squares represent auricular, the lower rectangles ventricular systole. The line uniting the two by its acuteness or obtuseness indicates the normal or a prolonged conduction time.

SINUS ARRHYTHMIA.

Whenever as in Fig. 2,B there is a pronounced irregularity of the radial tracing we must determine whether the arrhythmia is due to disturbances affecting the sinus rate, the so-called respiratory and phasic arrhythmias of vagus origin, or to definite pathological changes in the orderly sequence of events.

Proceeding with our analysis, we note that the "a," "c" and "v" waves occur with the usual time relationship to each other. There is no delay in conduction time, the "a-c" interval measuring less than 1/5 sec. There are no extra "a" waves present and there is no evidence of the occurrence of premature beats. There is, however, a difference in the length of the diastolic pauses due to the variation in rate at which the contraction impulse is set free.

SINUS BRADYCARDIA.

In the presence of a slow and regular pulse rate as in Fig. 2,C the possibility of a certain degree of heart-block must be kept in mind. This is excluded by the absence of any disturbance in the orderly spacing of the three "a."

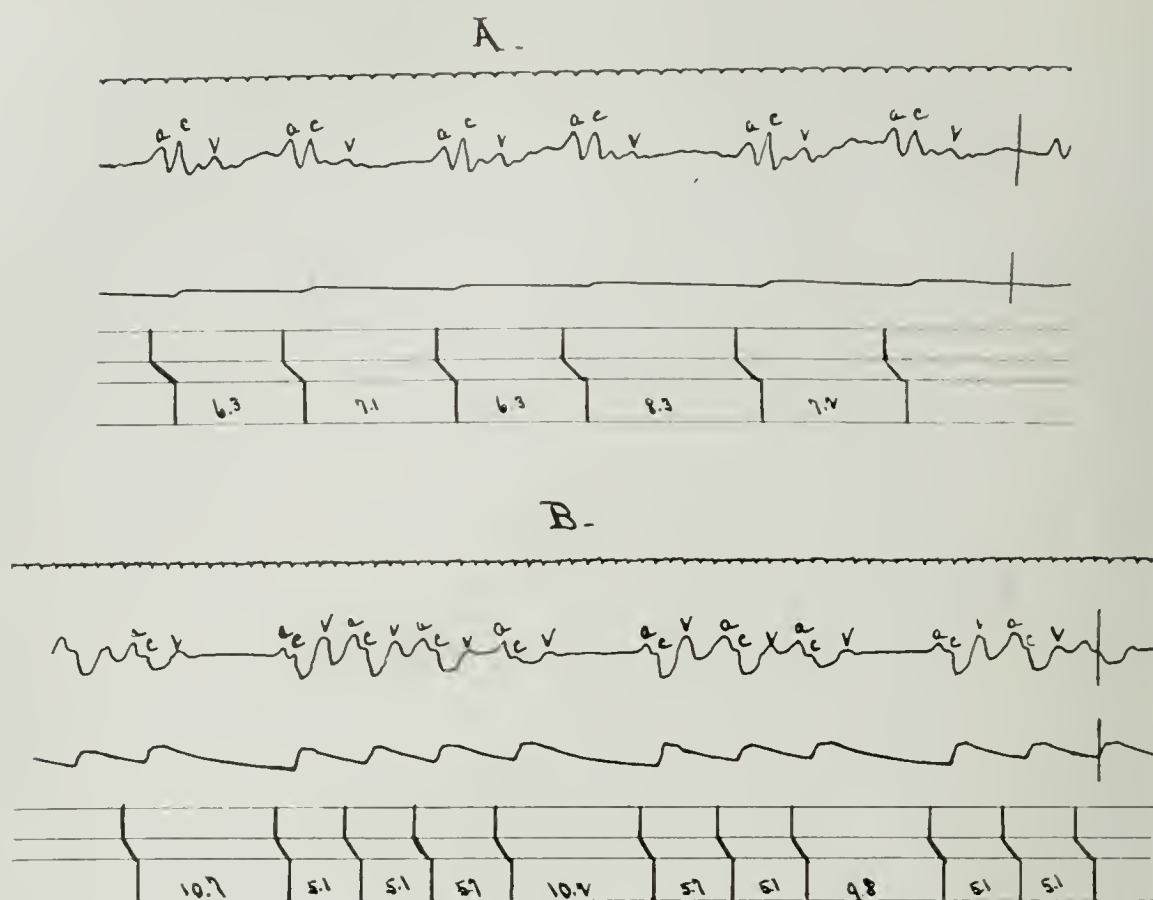


Fig. 4.—A. Marked sinus bradycardia, together with sinus arrhythmia. Pulse rate 38 to the minute. No delay in conduction time. Confirmed by electrocardiograph. B. Interpreted as sino-auricular block, associated with sinus slowing and arrhythmia. Pulse rate 58 to the minute. Confirmed by electrocardiograph.

“c” and “v” waves. The “a-c” interval measures less than $1/5$ of a second. There are no extra “a” waves present. Note the conspicuous rise in mid-diastole that may possibly be regarded as a “g” wave. There is no marked arrhythmia. We have to do with a simple sinus slowing.

In the instance illustrated in Fig. 4,A, we are dealing with an extreme degree of sinus slowing together with a sinus bradycardia. The “a-c” interval measures a full $1/5$ sec. Confirmed by the electrocardiograph. Note the split “v.” There is no disturbance in the orderly sequence of “a,” “c” and “v.”

SINO-AURICULAR BLOCK.

Whenever an apparently regular pulse is interrupted by pauses of considerably greater length than the normal interval between beats, the disturbance is commonly due to a premature beat which fails to carry through to the wrist, or to the failure of ventricular response giving rise to a dropped beat. The long pauses seen in the tracing in Fig. 4,B are due to an entirely different cause.

This tracing confirmed by electrocardiographic study has been interpreted as illustrating a true so-called sino-auricular block. There are no additional “a” waves present during the long pause in the jugular curve and there is no disturbance in the time relationship of “a,” “c” and “v.” The dominant rhythm in this bit of tracing and in additional long records spaces at 5.1. The first long pause at the left in the tracing measures 10.7, the second 10.2 just double the time of two beats of the dominant rhythm, and the third 9.8. The average dura-

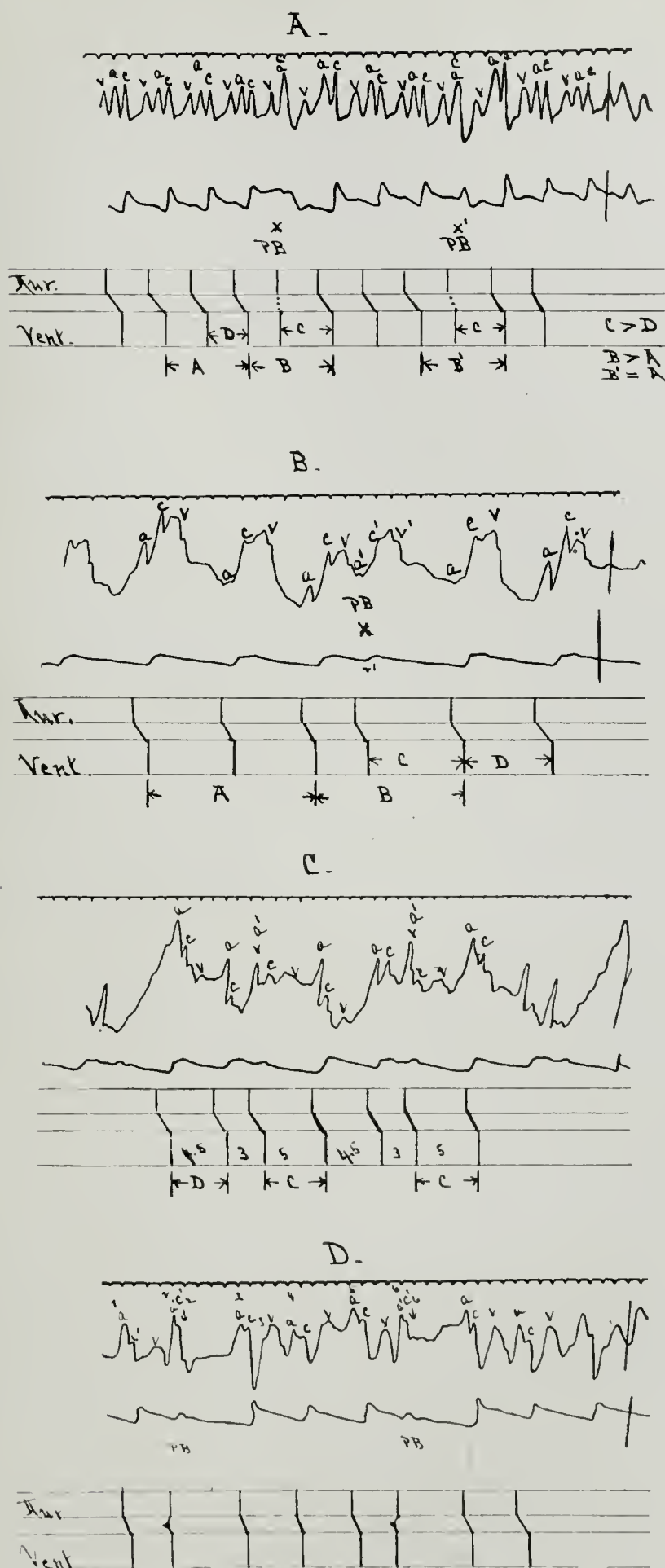


Fig. 5.—A. Premature beats ventricular in origin.
 B. Premature beats auricular in origin.
 C. Multiple premature beats, auricular in origin.
 D. Premature beats, possibly nodal in origin.

tion of the long pauses in many additional records measured 10.2 exactly twice the time of the dominant rhythm.

For some cause, at these long pauses, the formation of stimulus production takes twice the time normal for this individual.

PREMATURE BEATS.

When the normal rhythm is disturbed by one or more such irregularities as appear in Fig. 5,A the clue to the cause of the disturbance is at once suggested by analysis of the radial tracing alone and readily confirmed by a comparative analysis of the jugular and radial curve.

In this jugular tracing the "a" waves will be found to space regularly throughout. The "c" waves space regularly up to the point of disturbance marked x. At this point there occurs a premature contraction arising in the ventricle and the "c" wave corresponding to the accompanying radial coincides with an "a" wave, which occurring at its expected time finds the ventricle in a refractory state. Following this premature contraction there is a pause in the radial tracing longer than the time of one normal beat. This is called the "compensatory pause."

The period C is longer than D. With the first premature beat marked x, the period of disturbance—that period including the premature beat and the normal beat immediately preceding it—is slightly greater than the distance between two normal beats. With the second premature beat x' the period of disturbance B' is exactly equal to A. The reason for this compensatory pause is made clear in the accompanying diagram (Fig. 7,A). The premature contraction arises in the ventricle. The next ventricular response must wait for the next auricular systole. Note the absence of any disturbance of auricular rhythm, also the effect of the summation of "a" and "c."

When the premature contraction arising in the ventricle is so weak that it does not carry through to the radial, we have a pause in the radial curve equal to twice the time between two normal beats. A similar pause may be due to other causes. (See Figs. 4,B and 11,B.)

When following an irregularity in the normal rhythm the period of disturbance measures less than the distance between two normal beats and the compensatory pause C is longer than one normal beat, the premature contraction arises in the auricle.

In Fig. 5,B the "a" waves space up to the time of the disturbance. At this point the normal rhythm is interrupted by a premature beat marked x. We find "c" in relation to its corresponding radial wave r', and just before it, with the normal time relation, the "a" wave of the premature auricular contraction marked "a" in the figure. Following this premature contraction arising in the auricle there is a slight disturbance in the subsequent auricular rhythm and the appearance of the next "a" wave is a little delayed. Note that the period of disturbance B is less than A, while the compensatory pause C is longer than D.

When the normal rhythm is disturbed by multiple premature beats as in Fig. 5,C, and the compensatory pauses, or "end" pauses, are all of the same length the premature contractions are of auricular origin, excepting only that rare condition when the premature contraction may originate in the junctional tissues.

It occasionally happens, as in Fig. 5,D, that it is extremely difficult to de-

etermine by the polygraph alone whether the premature contraction arose in the auricle or in the junctional tissue. In the above figure "a²" and "a⁶" marked a' occur prematurely, by a short interval. "C²" and "c⁶" also occur prematurely and coincide with "a²" and "a⁶." The synchronous onset of "a" and "c" prematurely can only result from an impulse arising in the junctional tissue at the A-V node. The above tracing may possibly be regarded as illustrating premature contractions nodal in origin.

Whenever an irregularity repeats itself and the measurement of one cycle of the arrhythmia can be superimposed accurately upon a similar cycle appearing elsewhere in the tracing, the irregularity may be due to: (a) premature con-

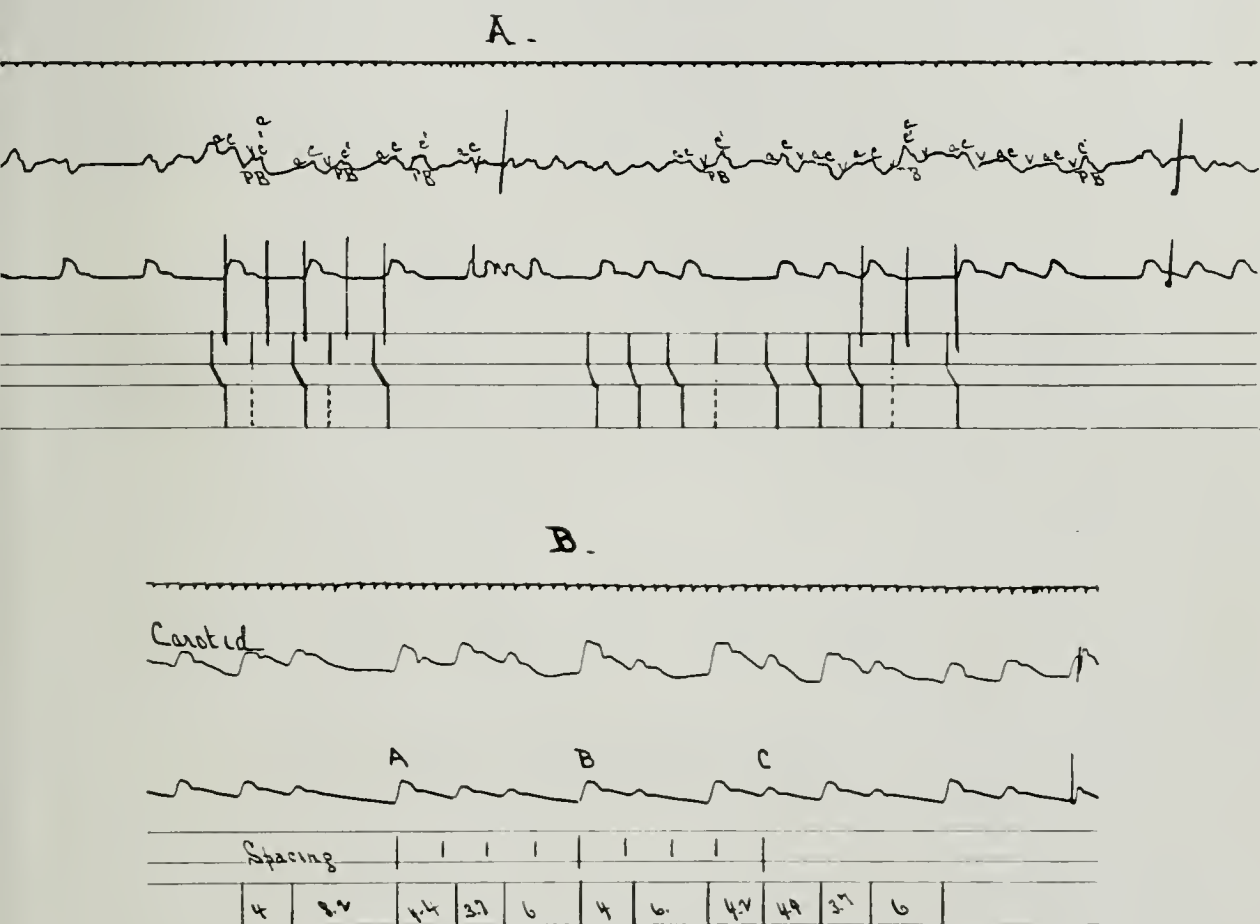


Fig. 6.—A. A regular irregularity due to premature beats ventricular in origin.
B. An irregularity due to premature beats ventricular in origin.

tractions ventricular or auricular, (b) to the occasional occurrence of dropped beats, or (c) to the presence of a high degree of heart-block with a wide variation in the ventricular response. No matter how irregular such a tracing may appear, if the irregularity repeats itself, or the radial can be spaced it cannot be due to auricular fibrillation.

Fig. 6,A illustrates a regular pulse alternating between a rhythm one-half the normal rate and a trigeminal pulse. The long pauses are twice the length of the short. The tracing can be spaced. It is due to premature contractions arising in the ventricle too weak to affect the radial.

In Fig. 6,B we have a type of irregularity frequently confusing. On careful measurement there is a wide variation in the length of the individual beats in the radial curve, and yet this tracing can be spaced. The period from A to

B equals that from B to C. The distance A-B and B-C is but a multiple of the shortest interval 3.7. In spite of the gross irregularity the pulse is not totally irregular. It is due to premature contractions arising in the ventricle.

PAROXYSMAL TACHYCARDIA.

When with a regular pulse the rate is altered abruptly, the change may be due to one of three causes.

If the change in rate bears a definite ratio to the previous rhythm and the pulse rate is exactly halved, the slow rate may be due to either premature ventricular contractions which do not reach the radial, or to the sudden development of a 2:1 heart block. When there is no exact ratio between the slow and the rapid rate the change is due to the sudden onset, or offset, of a paroxysmal tachycardia.

Fig. 8, A illustrates two short attacks of paroxysmal tachycardia. After a similar preceding paroxysm there was a return to the normal mechanism for one

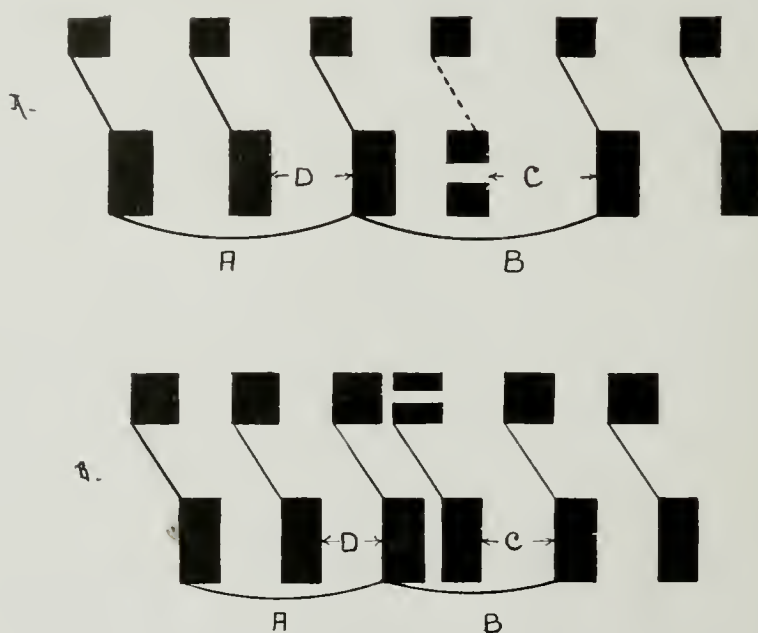


Fig. 7.—A. Diagram illustrating the reason for the disturbance in premature ventricular contractions. Note that C is greater than D, and that B equals A.

B. Illustrates the reason for the disturbance in premature auricular contractions. Note that C is greater than D, but that B is less than A. The split rectangle and square represent a premature contraction in this and all similar diagrams.

beat. At x another paroxysm onsets abruptly, the first beat of the new rhythm following a premature auricular contraction. The disturbance lasts for seven beats, there is a return to the dominant rhythm for one beat which in turn is again followed by a paroxysmal cycle lasting for seven beats. In long tracings from this case the attacks varied from seven to twenty beats. Such attacks may last from a few minutes to hours or even days. In the analysis of the jugular tracing the clue to "a," which after the first premature contraction falls with "v," is found in the initial beat of the new rhythm. Note that the end pauses are equal and that A equals B.

HEART-BLOCK.

When we can show in the jugular tracing that the "a-c" interval exceeds one-fifth of a second, it is evidence that there exists a delay in the conduction time.

This condition is called the first stage of heart-block. In Fig. 9,A, the "a-c" interval measures 1.2 fifths, exceeding slightly the normal of .2 seconds. This tracing also shows two premature auricular contractions.

When there is an occasional failure of the ventricle to respond to the impulse coming from the auricle it is called the second stage of heart-block or the stage of dropped beats. Fig. 9,B illustrates the occurrence of dropped beats. Note the prolongation of the conduction time up to the point of failure of the ventricular response. Note that the period B measures less than A.

In complete heart-block the analysis of the tracing is carried out in the usual way. Having determined "c" we may proceed to fix "v." We are at once struck by the variation in the general outline of "v." We then see that there is no wave present having the constant time relation of "a" to "c," but find a series of *regularly recurring* waves falling either before, with or after "c" and "v" in an apparently utterly haphazard relation to these two waves.

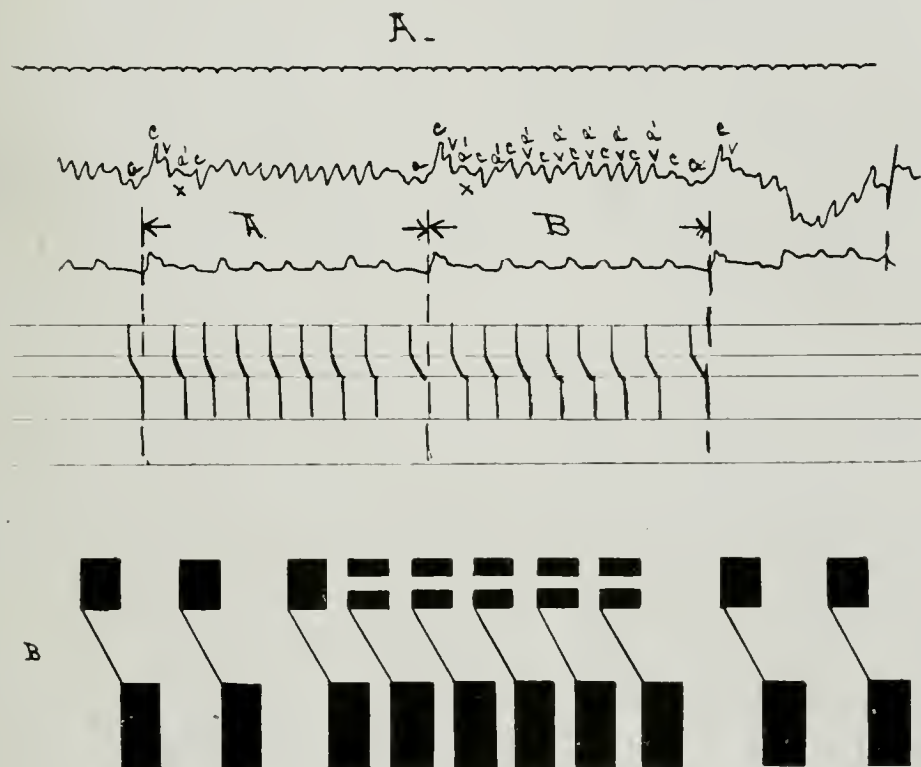


Fig. 8.—A. Paroxysmal tachycardia.

B. Diagram illustrating the nature of the disturbance in paroxysmal tachycardia.

The only possible source of a regularly recurring wave in the jugular curve at a higher rate of speed than "c" is auricular systole; if therefore these peaks can be shown to space accurately they are "a" waves. Note the difference in the summation of "a" and "c" and "a" and "v" in Fig. 9,C.

AURICULAR FLUTTER.

Although the polygraphic records of auricular flutter are often difficult to interpret, there are many instances in which the analysis is so simple that there should not be the slightest question as to the nature of the disturbance, while without them or without electrocardiographic records the diagnosis is impossible.

The jugular curve falls briefly into two types: one made up of a sequence

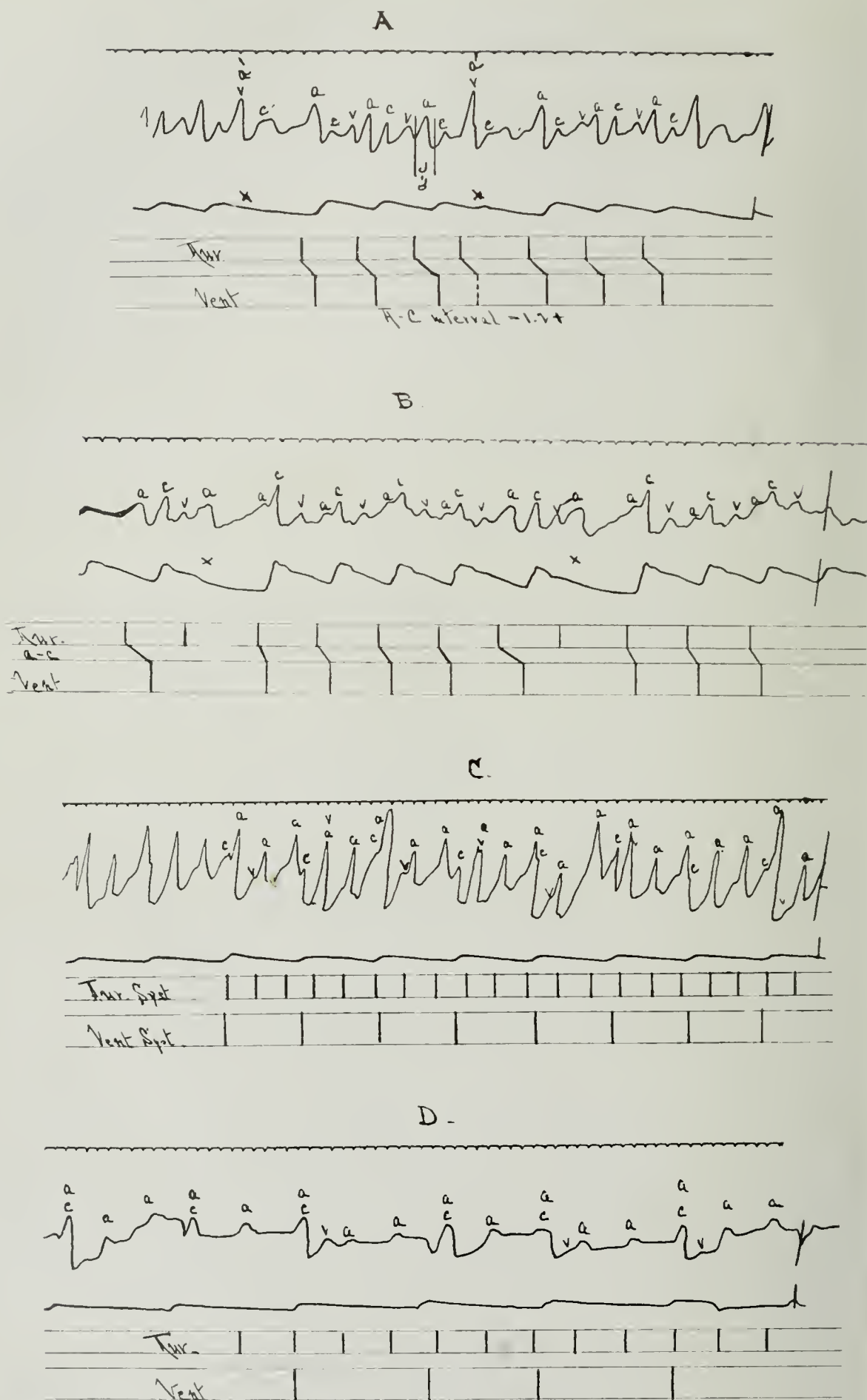


Fig. 9.—A. Delayed conduction time. A-C interval measures more than .2 seconds. First stage of heart block.
 B. Dropped beats. Second stage of heart-block.
 C. Complete A-V dissociation. Complete heart-block. Pulse rate 38 to the minute.
 D. Complete heart-block. Pulse rate 32 to the minute.

of rapid more or less uniform waves, the other appearing as paired waves with a constant time interval between the pairs. The pulse may be regular or irregular. Whenever there is present in the jugular tracing a regularly recurring sequence of waves, which space accurately from one cycle to the next, occurring at a rate higher than normal, with or without conspicuous summation auricular flutter should be suspected.

In Fig. 11,A, a tracing from a young individual with auricular flutter, the interpretation is evident. Note the conspicuous summation of "a" and "c." The distance A when transferred to any part of the jugular curve will include eight "a" waves. The ratio of ventricular response in this case was almost constantly 4:1.

In Fig. 11,B the analysis is more difficult. The radial tracing shows an irregularity, and the waves in the jugular curve are not conspicuous, though on close inspection a slight summation of "a" and "c" and "a" and "v" can be made out. A clue to the correct interpretation in this tracing is found in the regu-

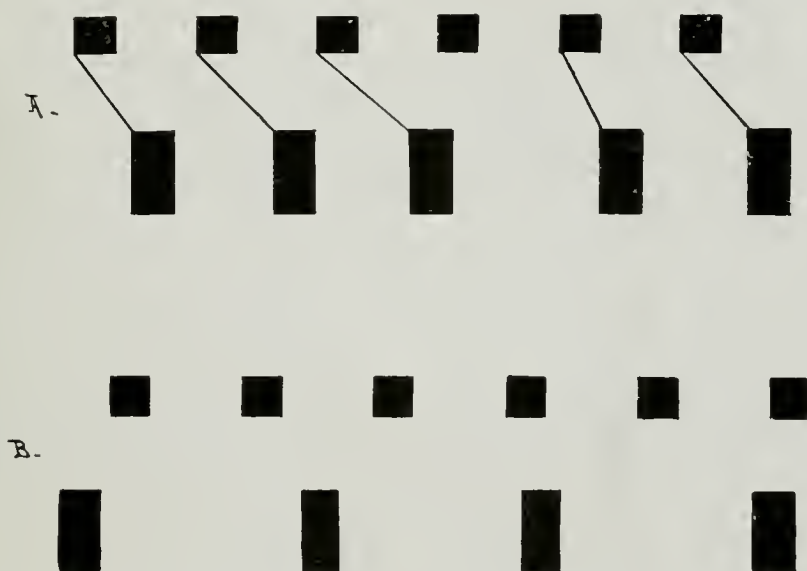


Fig. 10.—A. Diagram illustrating delayed conduction time and a single dropped beat.
B. Diagram illustrating complete A-V dissociation.

lar irregularity of the radial, the long intervals are exactly twice the length of the short. (See above for other causes of similar long pauses.) This fact together with the analysis of the jugular curve makes the interpretation simple. A given measurement on the radial tracing if transferred to any part of the jugular curve will include the same number of waves—auricular systole. In this tracing the period A, three long pauses, equals the period B, two long and two short pauses, and includes twelve "a" waves or auricular systoles.

When as sometimes happens we have an even more irregular response on the part of the ventricle we proceed in the same way to space the curve. In Fig. 11,C (from the same case) at the left of the tracing the ratio of ventricular response varied from 4:1, 3:1, 5:1. Here the distance A, 11 auricular systoles, equals the distance B, also 11 auricular systoles. At the time when this latter tracing was taken the predominant ratio of ventricular response was, however, 3:1. Note the summation of "a" and "c," and "a" and "v," and the constant time relationship of the paired waves, also that every third "a" wave lies buried

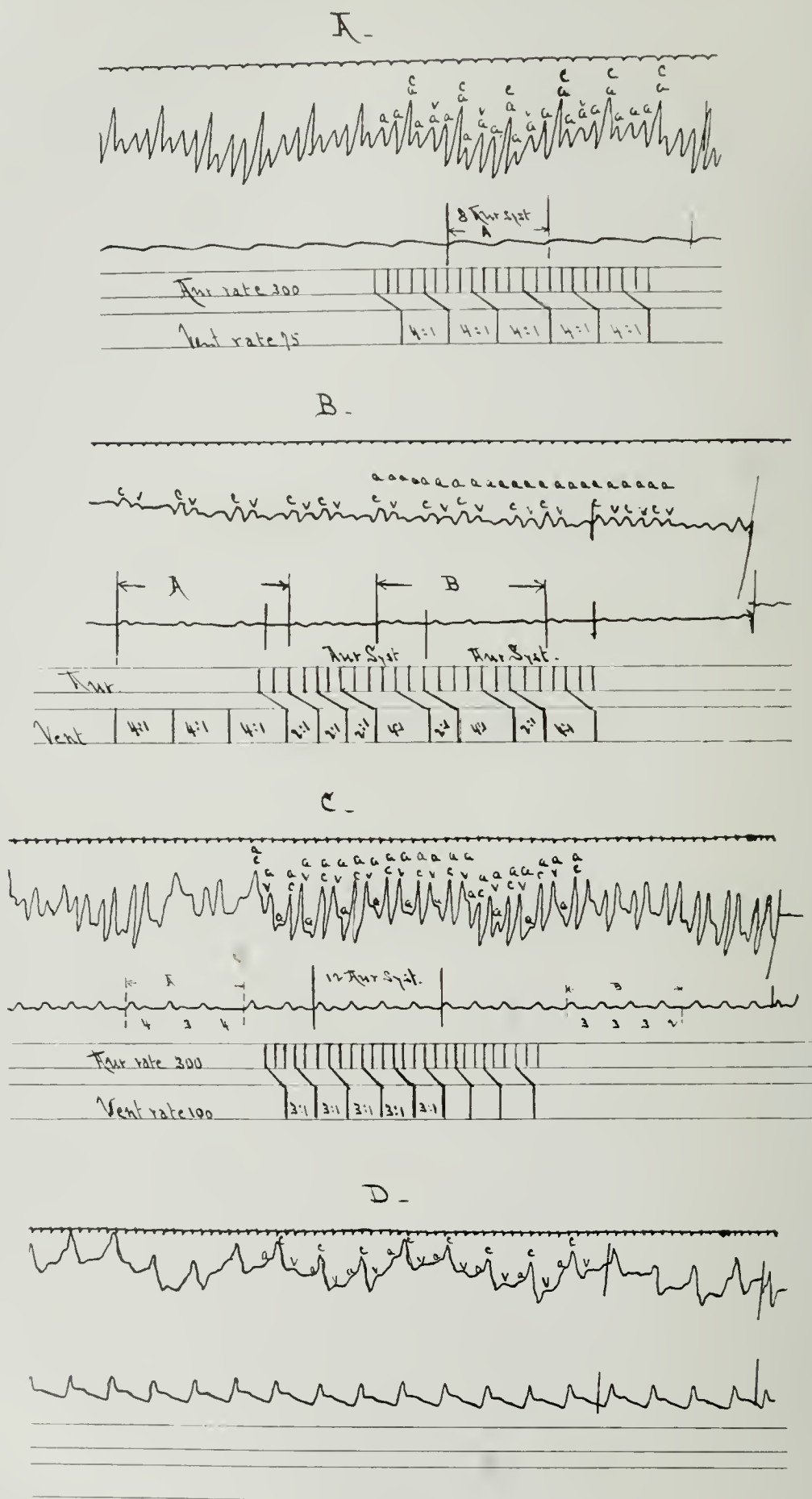


Fig. 11.—A. Auricular Flutter. Auricular rate 300, ventricular rate 75 to the minute.
 B. Auricular Flutter. Auricular rate 300, ventricular rate varied from 82 to 98 to the minute.
 C. Auricular Flutter. Auricular rate 300, ventricular rate 100 to the minute.
 D. Tracing from the same individual as B and C.
 Return to the normal rhythm following digitalis.
 Pulse rate 78 to the minute.

between "v" and "c" in this figure. Fig. 11,D is from the same case after restoration of the normal mechanism following digitalis. This case was confirmed by the electrocardiograph.

AURICULAR FIBRILLATION.

In auricular fibrillation the jugular curve is characterized by (a) the absence of all "a" waves; (b) the conspicuous summits of the jugular tracing fall during the sphygmic period, and (c) the occasional occurrence of "f" waves. The radial curve is characterized by (a) no evidence of a dominant rhythm; (b) the length of the individual radial beats varies from one cardiac cycle to the next; (c) the irregularity does not repeat itself; (d) the long pauses are not simple multiples of the shortest pause, and (e) the height of the radial wave frequently bears no relation to the length of the preceding pause. The pulse is said to be totally irregular.

The jugular curve constitutes the so-called ventricular form of venous pulse. Fig. 13,A and B illustrate typical examples of the tracings seen in this condi-

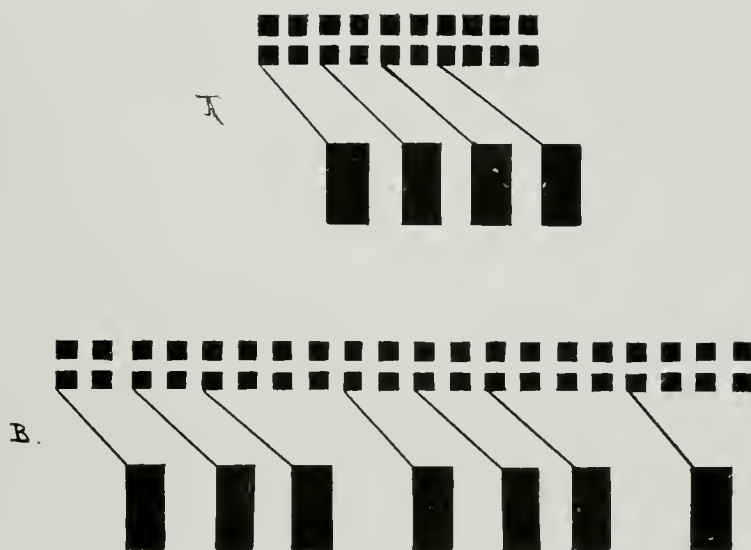


Fig. 12.—A. Diagram illustrating a 2:1 ratio in auricular flutter.
B. Diagram illustrating alternating ratios of ventricular response in auricular flutter.

tion; the latter figure showing the plateau type of ventricular venous pulse. The analysis is self-evident.

PULSUS ALTERNANS.

A true alternating pulse is one in which there is present a normal rhythm, the radial spaces accurately but the radial waves vary in height from one cardiac cycle to the next. It is regarded as of grave clinical significance.

RULE FOR CALCULATING THE PULSE RATE.

To calculate the cardiac rate from a given tracing, space off thirty 1-5ths on the time marker tracing; transfer this measurement to the radial curve placing the left hand end at the up-stroke of any radial wave. Beginning with this radial, as 1, count the number of beats, and fraction of a single beat, included within this measurement and multiply the number found by 10.

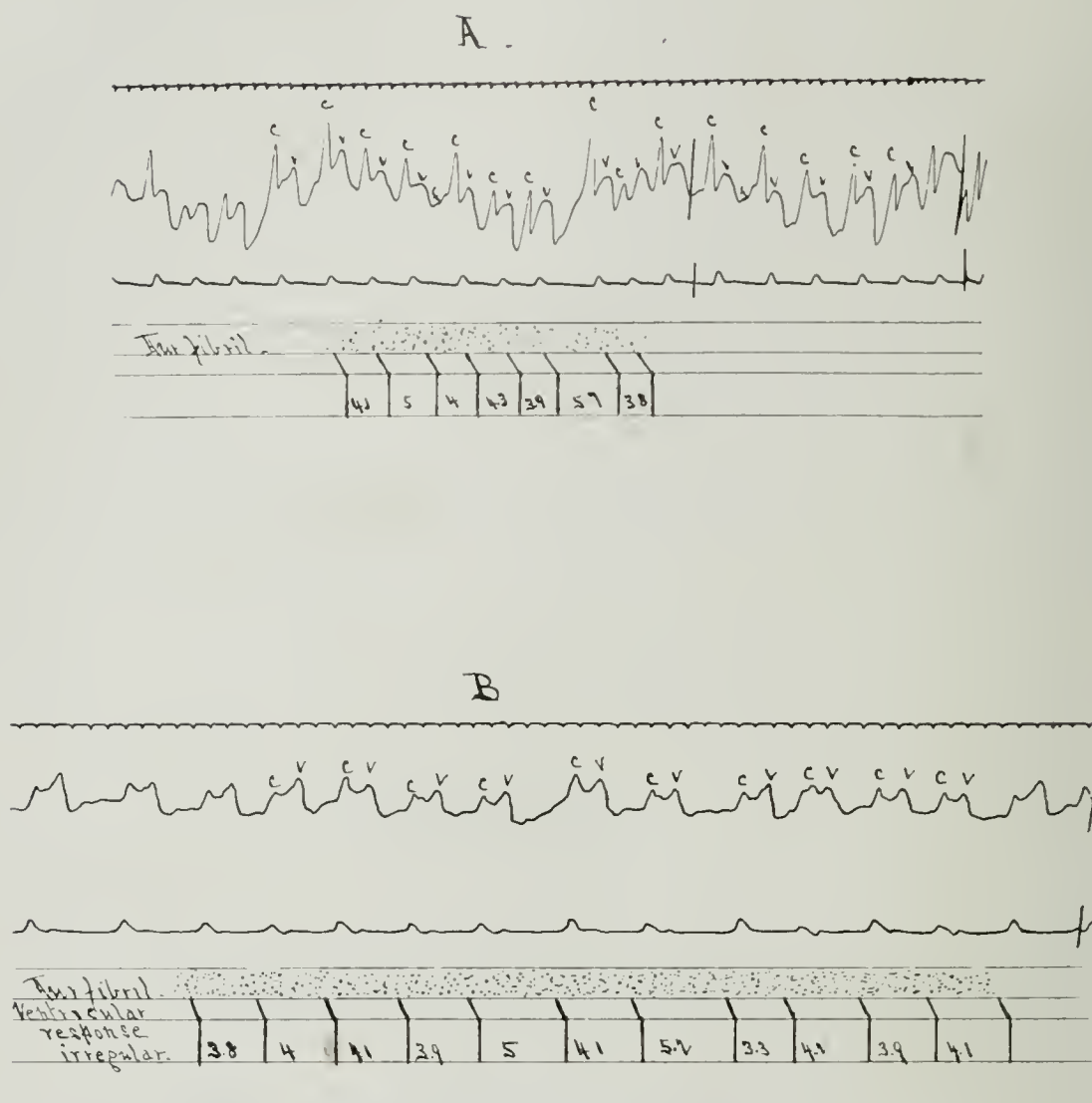


Fig. 13.—A. Auricular fibrillation. Note the absence of all “a” waves from the jugular tracing, the total irregularity of the radial curve and also that “c” and “v” fall during the sphygmic period.
B. Auricular fibrillation. Plateau type of venous pulse.

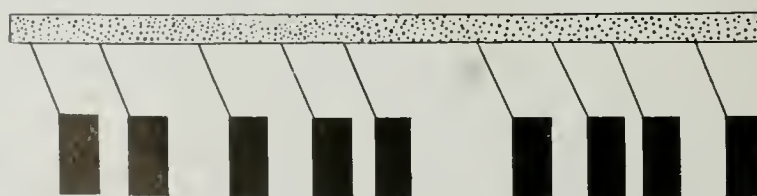


Fig. 14.—Diagram illustrating the nature of the disturbance in auricular fibrillation. Upper dotted space represents the auricles in the state of fibrillation. The ventricle responds at varying intervals to the haphazard impulses coming from the auricle.

CONCLUSION.

The fundamental principles of the polygraphic tracings and the method of procedure in their analysis have been described, as simply as possible. Their value as an aid to the proper understanding of many cardiac conditions has been emphasized, and their importance in the correct diagnosis of the cardiac arrhythmias insisted upon.

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THE DIAZO AND UROCHROMOGEN REACTIONS AS PROGNOSTIC AIDS IN PULMONARY TUBERCULOSIS*

BY H. J. CORPER, M.D., AND F. F. CALLAHAN, M.D., CHICAGO, ILL.

IN the hope of verifying or disproving the general conception regarding the prognostic value of the urochromogen reaction, and of comparing the value of Ehrlich's Diazo and Weisz' urochromogen tests, it was thought desirable to study these reactions in the urines of a large series of carefully observed tuberculous patients. The diversity of opinions reached from results obtained by a large number of observers also made this investigation desirable.

In reviewing the literature it seems hardly necessary to consider the diazo reaction of Ehrlich, since its value as a prognostic aid is so well established. The urochromogen reaction developed from a study made by Weisz¹ covering a period of about five or six years, which led him to the conclusion that there are two urochromogens or precursors of the yellow urinary pigment, one of these urochromogen α which can be oxidized by potassium permanganate, potassium persulphate, etc., into urochrom, the other, urochromogen β , which gives the diazo reaction of Ehrlich.

Schnitzker² who studied these substances from a chemical standpoint was able to separate and differentiate, although not quantitatively urochromogen α and β ; showing definitely that they were distinct chemical substances. Weisz in a further contribution states that the presence of urochromogen in the urine precedes the diazo reaction and answers all its purposes. He gives the ultimate history of twenty-three patients tested in this way, confirming the great prognostic value of this test. The earliest American observations on the urochromogen test, as far as we could find, were made by Heflebower³ who studied 3 cases of pulmonary tuberculosis and concludes that the urochromogen test is a better index as to prognosis than the diazo reaction; also that the intensity of these reactions is the important factor in the prognosis. Paranhos and Giolito⁴ studied the reaction in 50 healthy patients who always gave negative tests and 50 tuberculous patients which were all positive but 5; three of the latter being very far advanced. The reaction was not dependent upon the severity of the disease. In 50 cases suffering from other diseases (syphilis, nephritis, etc.) a positive reaction was obtained in 24. Vitri⁵ studied 150 cases of tuberculosis and found the reaction slight or absent in extrapulmonary lesions, also absent in incipient pulmonary cases running a chronic course. He also found the diazo reaction to be less sensitive than the urochromogen test. He concludes that a negative skin tuberculin reaction with a positive urochromogen indicates extension of the tuberculosis. 'Tuliato'⁶ found the urochromogen test positive in 67 per cent of all fatal cases in various diseases; it is not specific for any one disease; but is a sign of the gravity of the condition and explains it as due to the oxidation of albuminoid substances originating in the decomposition of cells as the result of direct or indirect action of pathogenic organisms.

*From the Laboratories of the Municipal Tuberculosis Sanitarium of the City of Chicago.

Weisz⁸ in a later article states that the appearance of urochromogen in the urine indicates that a local circumscribed tuberculous process has become generalized. The urochromogen disappears when the organism gets the upper hand, and the longer the reaction has existed the graver the prognosis. Cummings⁹ as a result of the examination of 100 cases concludes that the urochromogen and diazo reactions appear in the urine of a majority of the patients in a late stage of pulmonary tuberculosis. These reactions do not appear, however, until long after a correct unfavorable prognosis is possible by careful clinical examinations. Gullbring¹⁰ obtained only 2 cases out of 168 (mostly advanced stage of tuberculosis) in which the diazo reaction was positive and urochromogen negative, in 114 of these the findings were parallel, they were both negative in 88 and positive in 26. In all the others (37 per cent) the urochromogen was positive with negative diazo and these showed, besides the pulmonary lesions signs of amyloid disease. The urochromogen test was positive in 90 per cent of the amyloid cases while the diazo in only 20 per cent.

Metzger and Watson¹¹ examined 113 cases of tuberculosis with the urochromogen test and obtained negative results in 5 incipient cases, 32 per cent positive in 28 moderately advanced cases and 55 per cent positive in 80 far advanced cases, and conclude that the presence of the urochromogen reaction in the urine is of unfavorable prognostic import for the time being. Its persistent presence in spite of proper treatment probably means a hopeless prognosis. Its absence is generally, although not invariably, of good prognostic import regardless of clinical appearances. A prompt and continued disappearance is a favorable sign. Burgess¹² in a recent publication reports 469 urochromogen tests in 171 tuberculous patients and 1,030 tests on 650 non-tuberculous patients (suffering from other diseases) with but 26 positive tests in the latter which proved to be of no clinical significance. Fifty-five of the 171 tuberculous patients gave a positive test, and 30 of these died within three months, 6 within months and 1 within 1 year; the remainder were either worse, or observations discontinued. He concludes that the urochromogen test is of no value in determining the prognosis in non-tuberculous diseases; a positive reaction in advanced tuberculosis indicates progress of the disease with probable termination in 3 to 6 months, while a negative test is of no value. Shortly following there appeared a paper by Sinclair¹³ on the diazo and urochromogen tests in 146 cases of pulmonary tuberculosis. Both tests were negative in 38 cases of which 1 per cent improved, 18.4 per cent progressed and 10.6 per cent died. Both tests were positive in 7 cases, all of which died. In 23 cases the diazo reaction was positive with negative urochromogen and 25.7 per cent of these improved, 5.1 per cent progressed and 48.2 per cent died. In 78 cases the diazo was negative with positive urochromogen, 79.5 per cent of these improved, 15.4 per cent progressed and 5.1 per cent died. He concludes that a change from both negative to a negative diazo and positive urochromogen is more favorable than to a positive diazo and negative urochromogen. Both positives generally indicate an early death. A change from a positive diazo and negative urochromogen is favorable only on reversal (that is, to a negative diazo and positive urochromogen). If the urochromogen does not appear concomitantly with the disappearance of the diazo the prognosis is bad.

In order to observe whether there is any relation or difference between the occurrence of the diazo and urochromogen reactions in the various stages of pulmonary tuberculosis, the cases (350 in all) were studied after dividing them according to the National Association classification into incipient, moderately advanced and far advanced. To show further whether there was any relation between these reactions and the progress of the disease the examinations were classified depending upon whether the cases improved or not, giving two divisions as improved and unimproved. Since the study was to cover not only the prognostic value of both reactions but also the relation between the reactions and the value of each and both, it was necessary to note when the reactions were both present, individually present, or absent, and the changes that occurred on subsequent examinations. With these things in mind the cases were finally tabulated into the form shown in Table I.

As a result of the classification of the cases and reactions as shown in Table I it is to be noted that of the 82 incipient cases on whom 197 examinations were made, not one diazo reaction, whereas 8 positive urochromogens were obtained on initial examination; 4 of these 8 (2 of which were single examinations) improved and became negative, while 2 were unimproved and became

TABLE I.—CHANGE OF REACTION ON ADMISSION AND SUBSEQUENTLY. CLASSIFIED.

		D+U+	D+U—	D—U+	D—U—
Incipient cases, total exams. 197 on 82 patients	Initial Examination.			8 2-single exams.	74 25-single exams
	Subsequent Examinations, Improved.	D+U+			
		D+U—			
		D—U+			4
		D—U—		4	
	Subsequent Examinations, Unimproved.	D+U+			
		D+U—			
		D—U+			1
		D—U—		2	44
Moderately advanced cases, total exams. 256 on 112 cases.	Initial Examination.	4 4-single exams.	1 1-single exam.	12 6-single exams.	95 35-single exam
	Subsequent Examinations, Improved	D+U+			
		D+U—			2 1-with pleural effusio
		D—U+			3
		D—U—		6	35
	Subsequent Examinations, Unimproved.	D+U+			
		D+U—			2 1-with pleural effusio
		D—U+			3
		D—U—			15
Far advanced cases, total exams. 502 on 156 cases.	Initial Examination.	25 9-single exams.	5 2-single exams.	13 2-single exams.	113 21-single exam
	Subsequent Examinations, Improved	D+U+			2 1-with erysine
		D+U—	1		
		D—U+			2
		D—U—	6	5	22
	Subsequent Examinations, Unimproved.	D+U+	5	1	10
		D+U—	1	1	
		D—U+		1	3
		D—U—	3	2	53

negative. The remaining 74 gave negative reactions for both tests on initial examination, 18 of these improved, of which 14 had only initial examinations and 4 gave positive urochromogen tests on subsequent examinations; 56 were unimproved (11 of these were only single examinations), one of the remaining 45 gave a positive urochromogen on subsequent examination and 44 remained negative.

Of 112 moderately advanced cases with a total of 256 examinations 4 revealed both urochromogen and diazo positive, and one case gave a positive urochromogen and negative diazo (no subsequent examinations being made on these 5 cases). Twelve cases gave a negative diazo and positive urochromogen, 6 of which were only initial examinations, and 6 cases improved and became negative on all subsequent examinations. Ninety-five cases were negative to both reactions, 35 of these having only initial examinations; 40 cases improved of which 2 gave a positive diazo and negative urochromogen (one having had a pleural effusion), 3 revealed a negative diazo and positive urochromogen, and 35 remained negative throughout. Twenty of the 95 diazo negative urochromogen negative initial cases were unimproved, 2 giving a positive diazo and negative urochromogen (one of these had a pleural effusion), 3 cases a negative diazo and positive urochromogen, and 15 remained negative throughout.

Of 156 far advanced cases upon which a total of 502 examinations were made, 25 gave both reactions positive upon the first examination, 7 of these 25 improved (9 only had initial examinations) one of which became positive diazo and negative urochromogen and the remaining 6 became negative. There were 9 unimproved cases; 5 remained positive diazo and positive urochromogen, one became positive diazo and negative urochromogen, and 3 became negative to both reactions on subsequent examinations. Five cases gave positive diazo and negative urochromogen on admission, of which 2 were only initial examinations, and the remaining 3 were unimproved—1 of these remained positive diazo and negative urochromogen while the other 2 became negative to both tests. Of 13 cases which gave negative diazo and positive urochromogen on admission, 2 were initial examinations only, 5 improved and became negative to both tests and 6 were unimproved of which 1 became positive to both tests, one positive diazo and negative urochromogen, 1 remained the same and 3 became negative to both tests. Both reactions were negative on initial examination in 113 cases (21 of these being only single examinations), 26 improved—2 of which became positive to both tests (one of these during an attack of erysipelas), 2 became negative diazo and positive urochromogen and 22 remained negative on subsequent examinations. Of the 113 cases 66 were unimproved, 10 of which became positive to both tests, 3 became negative diazo and positive urochromogen and 53 remained unchanged on subsequent examinations.

The large number of negative reactions in the far advanced cases and an endeavor to explain these reactions made it seem advisable to further classify 103 far advanced cases into cases with far advanced physical signs and few symptoms, as far advanced fibroids, and those showing far advanced signs and active symptoms as far advanced active cases. The result of this classification is shown in Table II.

TABLE II.—COMPARISON OF REACTIONS IN FAR ADVANCED FIBROID AND FAR ADVANCED ACTIVE CASES.

			D+U+	D+U—	D—U+	D—U—
Far advanced Fibroid cases.	Initial Examination.		5 1-single exam. 3-acute exacerbations 1-spontaneous pneumothorax on admission	2	4	63 5-single exams.
	Subsequent Examinations.	D+U+				3 including 1-enteritis 2-acute exacerbations
		D+U—				
		D—U+			1	4
		D—U—	4	2	3	51
Far advanced Active cases.	Initial Examination.		7 4-single exams.	1	2	19 3-single exams.
	Subsequent Examinations.	D+U+	2		2	10
		D+U—	1			1
		D—U+				
		D—U—		1		5

In this table there were classified 74 far advanced fibroid and 29 far advanced active cases. Of the fibroid cases 5 were positive diazo and positive urochromogen on admission (one only having an initial examination) 3 of these 5 had acute exacerbations and 1 had acute pneumothorax when examinations were made; 4 became negative on subsequent examinations. Of the 74 fibroid cases 2 had positive diazo and negative urochromogen on admission and became negative to both tests; 4 had negative diazo and positive urochromogen on admission of which 1 remained the same and the other 3 became negative to both tests on subsequent examinations. There were 63 of the far advanced fibroid cases which gave negative to both tests on initial examination (5 of these being single examinations), 3 cases (1 developing tuberculous enteritis and 2 during an acute exacerbation) became positive diazo and positive urochromogen. Four changed to negative diazo and positive urochromogen and 51 remained unchanged on subsequent examinations.

Of the 29 far advanced active cases 7 were positive to both tests on admission (4 of these were single examinations), 2 remained the same on subsequent examinations and 1 became positive diazo and negative urochromogen. Only 1 of the 29 cases gave a positive diazo and negative urochromogen reaction on admission and subsequently became negative to both tests. Two cases were negative diazo and positive urochromogen on initial examination and became positive to both reactions subsequently. Nineteen cases gave a negative reaction to both tests on admission (3 of these being only initial examinations), 10 became positive diazo and positive urochromogen, 1 became positive diazo and negative urochromogen and 5 remained unchanged on subsequent examinations.

The terminal cases, of which there were 60, were tabulated separately in Table III.

TABLE III.—CLASSIFICATION OF REACTIONS IN TERMINAL CASES.

		D+U+	D+U—	D—U+	D—U—
Initial Examination		34 15-single exams.	6 3-single exams.	5 4-single exams. 1-terminal hemorrhage	15 3-single exams. 1-terminal hemorrhage
Subsequent Examinations 133 examinations. on 60 cases.	D+U+	14	2		4
	D+U—	2			
	D—U+	2 including 1-T. B. meningitis		1	3 including 1-terminal hemorrhage 1-acute peritonitis
	D—U—	1 including 1-T. B. meningitis	1		5 including 2-T.B. meningitis 1-terminal hemorrhage 1-acute lobar pneumonia 1-acute cardiac

Of these 60 terminal cases 34 gave a positive diazo and urochromogen reaction on initial examination (15 of these being only single examinations), 14 remained positive on subsequent examination, 2 changed to positive diazo and negative urochromogen and 2 changed to negative diazo and positive urochromogen, while 1 became negative. One of the 2 cases that changed to negative diazo and positive urochromogen died of tuberculous meningitis.

Six of the 60 cases were positive diazo and negative urochromogen on initial examination (3 of these were single examinations), 2 changed to positive diazo and urochromogen and one to negative diazo and negative urochromogen on subsequent examinations.

Five of the terminal cases gave a negative diazo and positive urochromogen on initial examination (4 being single examinations, one of which died of terminal hemorrhage), one showed no change subsequently. Fifteen of the terminal cases gave negative results on initial examination (3 of which were single examinations, one of these dying of terminal hemorrhage), 4 became positive to both reactions on subsequent examinations; 3 became negative diazo and positive urochromogen including one who died of terminal hemorrhage, and one from acute peritonitis due to a perforation of a tuberculous ulcer, and 5 remained negative [2 of which died from tuberculous meningitis, 1 from a terminal hemorrhage, 1 of acute lobar (pneumococcus) pneumonia, and one from cardiac insufficiency].

It is to be noted from these tabulated results of the findings in terminal cases that practically all cases dying of pulmonary tuberculosis reveal positive reactions by both tests at some time during the last 6 months of their disease. Those cases which gave negative reactions to both tests during the last 6 months of the disease usually died of some acute condition such as pulmonary hemorrhage or tuberculous meningitis, etc. It was noted that cases dying of tuber-

culous meningitis usually gave negative reactions to both tests; this was especially true of the diazo reaction. Some of these cases were negative during the whole course of the disease, others became negative during the attack of meningitis.*

CONCLUSIONS.

1. Cases dying of pulmonary tuberculosis give a positive diazo and urochromogen test at some time during the last 6 months of their illness. Whenever both tests are negative during this period death was found to be due to some intervening condition, such as pulmonary hemorrhage, tuberculous meningitis, etc.

2. Cases of chronic fibroid tuberculosis generally give both reactions negative except when same is explicable by some acute exacerbation, acute intercurrent infection or acute pleural effusion.

3. In active cases of pulmonary tuberculosis when both reactions are positive and remain so for most of the succeeding examinations it is of grave prognostic import. When both reactions are negative in acute cases no stress can be laid upon the findings.

4. There seems to be no regularity between the presence of either reaction, its disappearance to be displaced by the other or by both.

5. The presence of a urochromogen reaction in cases showing no clinical symptoms is of no prognostic value. No diazo reactions were obtained in clinically inactive cases, whereas a number of urochromogen reactions were obtained in such cases.

6. The presence of a diazo or of both reactions is a *danger signal* of grave import.

7. Careful clinical observations will, from a prognostic standpoint, give more information than the diazo and urochromogen tests in pulmonary tuberculosis.

8. Wherever possible both tests should be performed, but of the two the diazo should be given the preference.

*We wish to express our appreciation to Dr. M. I. Marshak for the kind assistance rendered in preparing this paper.

THE HOSPITAL CHEMICAL LABORATORY*

By N. W. JANNEY, M.D., Ph.D., New York City.

PRESENT STAGE OF DEVELOPMENT OF THE HOSPITAL LABORATORY.

THE development of the American hospital laboratory seems curiously enough to have lagged far behind the modern evolution of the medical sciences. The "laboratory" of even the large urban hospital of not so many years ago still very frequently presented little evidence of advance. A young physician often in active practice was frequently found in sole charge of the laboratory work,—bacteriological, chemical, pathological, and serological. His assistants were as a rule resident physicians who, bent on a purely clinical career, found the clinical side of medicine so engrossing that their laboratory duties frequently suffered as a consequence. Under these circumstances it is then not surprising that the newer delicate scientific methods of precision were inadequately carried out and that but relatively little original research of value resulted from such an organization. So the physical equipment of such a laboratory was as a rule but illy provided for or indeed neglected. This materially contributed to a generally unsatisfactory state of affairs.

In order to meet such needs many of the larger city hospitals, the institutions directly connected with universities being excepted throughout this discussion, have developed so-called "laboratories of clinical pathology" which represent about the present stage of evolution of the hospital laboratory. The head of such a laboratory who now more frequently devotes his entire time to his hospital duties, is usually a pathologist or bacteriologist and often intends following a wholly scientific career. This implantation of a purely scientific man into the hospital system represents a decided step in advance. Numerous examples can be quoted where research studies of important nature have been carried out under these circumstances. The "clinical pathologist" has become so generally recognized that in certain universities chairs of clinical pathology have been created. There is indeed no doubt that the research as well as routine in pathology, bacteriology and serology done in many large hospitals can now be said to be entirely on a par with the modern development of these scientific medical branches.

NEED OF A HOSPITAL BIOCHEMICAL LABORATORY.

At the present time we are however in the midst of a great era of expansion of chemical knowledge as applied to medicine. It can only be likened to the previously occurring rapid development of bacteriology when its possibilities became clearly recognized. Within the past two decades the pure chemists, headed by Emil Fischer, have made such advances in the field of physiological chemistry, animal and human, that the combined work of all previous time has been surpassed. No other example than protein chemistry need be considered in order to emphasize the truth of this assertion.

Some time back the physician trained in clinical pathology could with no

*From the Chemical Laboratory of the Montefiore Home and Hospital for Chronic Invalids, New York City.

great effort include among his duties the chief chemical examinations then deemed advisable to be carried out in the hospital laboratory. Such procedures as compared to the total work of the laboratory were few and usually required no unusual technical skill of chemical nature. This state of affairs has, however, changed considerably of late. Newly introduced biochemical methods are becoming ever increasingly such direct aids to clinical diagnosis and treatment that of the total clinical laboratory examinations a large percentage are already chemical. The newer methods for the quantitative determination of various constituents of the blood may here be mentioned as an example. In many instances a greater knowledge of chemistry and technic is also required in order to obtain accurate analytical results by these new methods than can be fairly expected of the physician with his necessarily general training, without undue loss of time and energy.

The prevailing type of hospital laboratory, as alluded to above, certainly seems but poorly prepared to cope with these new demands made on it by biochemistry. In organization essentially medical from director down, there is frequently no one connected with it on whom chemical examinations of a complex nature can properly devolve. This fact was very forcibly impressed on the writer in a comparative study recently made by him of hospital laboratories. Frequently first rank institutions were found to possess experienced pathologists, serologists and bacteriologists quite competent to fill higher university positions, whereas it is a matter of great rarity that the biochemist, when such a post is actually existent, possesses such qualifications. Indeed only a very few existing instances are known of hospital chemical laboratories developed commensurately with the position now attained by biochemistry among the medical sciences.

IMPORTANCE OF THE HOSPITAL CHEMICAL LABORATORY FOR BIOCHEMICAL RESEARCH AND TEACHING.

In considering the relations of chemistry and medicine of today a certain lack of perspective is often observable in the work of both chemists and physicians engaged in the physiological chemical field. As extreme examples the following two episodes may be quoted. A certain large city hospital in Germany, deciding on modernization, secured the services of an organic chemist who was instructed to enter into research studies of clinical importance. This worker knowing that hemoglobin is an important blood constituent spent two years and would have probably spent many more if he had been permitted, in working on *the configuration of the hemoglobin molecule*. The vague relation of this research to clinical medicine is quite obvious.

The second incident is of the opposite nature. A young physician secured a position to do research work in biochemistry. With the intensest devotion and application he succeeded after a year in introducing a single quite unimportant modification into a standard biochemical analytical method. This work, however creditable to its author in view of his limited chemical training, can hardly be said to represent an adequate return of the time and money involved. But such illustrations of inexperience are at least useful in emphasizing a criticism which may be made of present day biochemical research and teaching. Too often an unfortunate one-sidedness is apparent in the biochemical research

worker or teacher. This tendency to uneven development may be either in the chemical or medical direction, but usually the chemical is the predominating. As a result the purely chemical side of this truly border subject is often rather over dwelt upon. Indeed the research studies of certain university departments of physiological chemistry would do like honor to a university chemical institute. Owing to the same one-sidedness, medical students are not always sufficiently drilled in those portions of biochemistry most useful to their careers as practitioners.

The underlying cause of these difficulties lies in the fact that it is not easy to acquire the ideal blending of chemical and medical experience most desirable for research workers and teachers of biochemistry. The pure chemist has far too few opportunities in the university department of physiological chemistry to come into needed contact with the clinical side of medicine. Conversely if beginning his biochemical career as a physician and receiving his introduction to biochemistry in a hospital laboratory, the fundamental chemical training of this future investigator and teacher of biochemistry often remains permanently inadequate.

If, however, our large hospitals possessed efficient chemical laboratories conducted by biochemists of broad experience, they could serve as nearly ideal training places for future workers in this field. Here the young chemist could come into daily contact with medical internes as well as patients, learn something of morbid conditions at first hand, and in general enjoy a peculiarly rich opportunity for acquiring much of the medical knowledge necessary for his future development as a biochemist. After some years spent in this atmosphere he could then accept a position in a university department of biochemistry with every prospect of keeping a proper balance between the chemical and medical side of his subject.

Another reason may here be mentioned for the need of development of hospital biochemical laboratories. It is in the hospital and not so frequently in the university departmental biochemical laboratory as at present that the biochemist should also find material for original investigation. The writer in delving through medical literature has many times been impressed by the advances which could have been made had many questions been studied by biochemists rather than been left to their purely medical confreres, whose enthusiasm for objects of chemical research frequently far surpasses their chemical attainments. If the biochemist had been at hand in the hospital many of such investigations would have had higher value.

RELATION OF THE CLINICAL STAFF TO THE HOSPITAL BIOCHEMIST.

A very unfortunate arrangement is frequently met with in hospitals at present. Owing to shortness of funds or to a lack of realization of the value of an experienced biochemist to the institution, a young worker in this field is frequently placed in charge of the chemical laboratory. Such an incumbent very frequently fails of proper development, this being due to his premature isolation and separation from older biochemical colleagues. Another patent cause for the lack of success of hospital laboratories may also play a role, namely a too great control of the work, especially of research, on the part of the clinical authorities. This may be necessary in the case of the

young and inexperienced chemist who might otherwise fail to devote himself to well judged research problems. However in many otherwise well organized hospitals the belief seems still to persist that the control of the research activities of the chemist and indeed also of all branches of hospital laboratory research should lie with the clinical staff. It is very surprising that such an obviously detrimental arrangement should, in view of the modern development of medicine, require much discussion. Objectively considered it seems no more reasonable that the experienced original investigator in the scientific medical branches should have his problems selected for him by the clinician, than it would be for him to attempt to prescribe changes in the treatment of the hospital patients under control of the visiting physicians. It is, however, as fully recognized by the writer with regard to his own subject that only to one of broad training both in medicine and chemistry can such independence be safely given in choice of hospital laboratory problems. In this lies an additional argument for furthering the development of a type of original investigator in biochemistry having the hospital training suggested in this article.

What then constitutes the most judicious relation of the visiting physician to the hospital biochemist? Prof. Frederick Müller of München once expressed to the writer the conviction that the "*Fragestellung*" should be the aim of the clinician with regard to biochemical research. The discerning bedside physician coming into intimate contact with the innumerable variations of morbid phenomena has indeed many occasions to formulate questions often of extreme interest and importance to the biochemist. It remains for the latter, however, to judge of the feasibility of a chemical attack on such questions and to devise means for the solution of such as lend themselves to elucidation. Again many other problems can best be studied by the clinician, biochemist, and at times also the pathologist working in common. Many valuable researches of recent years have been made possible only by such an efficient coalition of specialized knowledge and experience.

ORGANIZATION OF A HOSPITAL BIOCHEMICAL LABORATORY.

Providing that an adequately trained and experienced biochemist is obtainable this should present no great difficulty. As previously discussed it is at present quite rare to find men sufficiently versed in both medicine and chemistry to become ideal incumbents of such positions. To its holder are frequently referred problems involving very considerable knowledge of clinical medicine. On the other hand the solution of many questions may tax all the resourcefulness of an able chemist. The work of the director of such a laboratory can be best carried on with the help of an assistant chemist, who also receives medical experience as suggested above. If funds permitted, other assistants could be employed for research problems only. One or more technicians for the simpler chemical routine such as urinalysis and gastro-analysis could complete the staff.

In hospitals affiliated with universities the director of the hospital chemical laboratory could likewise hold an assistant or associate professorship in the teaching institution. Certain biochemical students preparing themselves for their doctor's degree could then be given an opportunity for the working out of their theses in the laboratory. In this way, also, it is possible to more evenly blend medical with chemical experience in the development of the future biochemist.

As to the physical equipment, four categories should be considered. (1) the chemical laboratory, (2) the metabolic ward, (3) provision for animal experimentation, (4) library. For the hospital chemical laboratory to meet the requirements suggested in this article very judicious equipment is necessary. The routine chemical analyses of urine, blood, gastric fluid, etc., can best be carried out in a separate room. Other space should if possible be reserved for purely research studies. This division is but rarely seen as yet, but is of considerable importance to the workers. As the problems and therefore the chemical operations carried out in this laboratory vary constantly, it is very important to install only such apparatus as permits of being easily changed about to meet the shifting requirements of the work. In an article of this kind it seems inadvisable to go into details of this and a number of other very practical matters of similar nature.

As a matter of common experience, it is very difficult to properly carry out metabolic examinations and experiments in the midst of an active medical ward. Segregation of patients undergoing such studies as well as their complete control is essential for the proper conduct of such work. For most requirements two small rooms for male and female patients with a conveniently situated special diet kitchen are adequate. Too much care can scarcely be given to the training of the dietician and nursing staff of such an experimental ward, inasmuch as the entire value of a long and laborious metabolic study is directly dependent on the faithfulness and accuracy of the dieting of the patient and collection of the excreta. The location of this ward should if possible be directly adjacent to the chemical laboratory.

As the animal rooms available to the biochemist in a hospital are scarcely ever set aside exclusively for his use, their equipment must depend somewhat on the needs of the other scientific workers. In general the plan adopted by the Stadt Krankenhaus at Frankfurt am Main, Germany, seems a good one and may be mentioned. Rooms for dogs and smaller animals, a room for experimental procedures, such as catheterization requiring at most simple sterile precautions, together with a well furnished aseptic animal operating room and ante-room enter into the makeup of this plant.

Provision for a working library even if necessarily very restricted in size should be made in such a complete laboratory development. Very few hospital libraries in America known to the writer are in any way adequate to the calls made upon them by the various workers. The reason often given in explanation of such a deficit in ready-to-hand knowledge is that such books are too expensive to acquire. As a matter of fact one thousand dollars judiciously invested by a competent authority suffices to obtain a very useful working set of journals and text-books in any one of the scientific medical branches. When it is considered that the mechanical equipment of the American laboratory is often more than adequate for its purposes and unnecessarily expensive as well, it would be wiser, the writer believes, to devote to library purposes some of the money often represented by little used pieces of large apparatus and such luxuries as plate glass shelves.

Inasmuch as the general arrangement of the laboratories of the new Montefiore Home and Hospital has been demonstrated through several years of use

to be convenient, mention may be made of their general plan. One of the buildings belonging to the hospital complex is occupied by male and female medical wards on its first two floors. The third floor of this building is occupied by the laboratories adjacent to which is a small experimental ward, diet kitchen and the research library. Immediately over the laboratory floor are built the animal rooms. This close physical association of the various elements going to make up the laboratories has proven of considerable advantage.

THE NINHYDRIN COLOR REACTIONS OF PROTEINS AND THEIR SPLIT PRODUCTS*

BY HERBERT W. EMERSON, M.D., AND JOHN S. CHAMBERS, M.S.,
ANN ARBOR, MICH.

THE NINHYDRIN REACTION.

THE ninhydrin color reaction^{2, 3} was first tried on the poison cleaved from casein. The poison was made up in aqueous solutions (1-100); (1-1,000) and (1-10,000). The acidity was determined and neutralized with N/10 sodium hydroxide. The color test was performed by taking 1 c.c. of this solution in a small, narrow test tube and adding one drop of a one per cent ninhydrin solution and placing it in a rack in an oil bath kept at 120° C., and leaving it there for two minutes; then the tube was removed and permitted to cool. The solution (1-100) gave a light reddish-violet color; the solution (1-1,000) gave no color to the liquid but deposited a blue line upon the sides of the test tube at the surface of the solution. The solution (1-10,000) was negative, no color and no ring. This experiment was controlled by glycocoll solutions of similar strengths. Glycocoll solutions (1-100) and (1-1,000) gave a deep blue color and the solution (1-10,000) gave a very pale blue color which deepened some upon standing for one hour and then gradually faded.

We tested in the same way poisons cleaved from colon bacilli; from the bacilli of tuberculosis; from dog's muscle; from dog's liver; and from beef's kidney; and the results were as they are described in the casein poison with slight variations in intensity of color.

The next series of experiments were performed in order to determine whether anything in the protein poison solution interfered with the ninhydrin reaction.

Experiment I was performed with casein poison solution (1-100) and adding glycocoll (1-100) and ninhydrin and heating as before.

TABLE I.

CASEIN POISON (1-100).	GLYCOCOLL (1-100).	1% NINHYDRIN.	RESULTS.
1 c.c. casein solution	+ 1 drop glycocoll	+ 1 drop ninhydrin and heat	—
1 c.c. " "	+ 2 drops " "	+ 1 " " " "	—
1 c.c. " "	+ 4 " "	+ 1 " " " "	—
1 c.c. " "	+ 8 " "	+ 1 " " " "	Weak +
1 c.c. " "	+ 16 " "	+ 1 " " " "	Fair +
1 c.c. " "	+ 32 " "	+ 1 " " " "	Good +

*From the Hygienic Laboratory of the University of Michigan.

This investigation was suggested and part of the work directed by Dr. V. C. Vaughan.

These experiments were performed on poisons cleaved from various proteins according to Vaughan's method.¹

This experiment was repeated but the casein poison solution was first neutralized.

TABLE II.

CASEIN POISON (1-100).	GLYCOCOLL (1-100).	1% NINHYDRIN.	RESULTS.
1 c.c. casein solution	+ 1 drop glycocoll	+ 1 drop ninhydrin and heat	—
1 c.c. " "	+ 2 drops " "	+ 1 " " " "	Weak +
1 c.c. " "	+ 4 " "	+ 1 " " " "	Fair +
1 c.c. " "	+ 8 " "	+ 1 " " " "	Good +

The above experiment was repeated using casein poison solution (1-1,000).

TABLE III.

CASEIN POISON (1-1000).	GLYCOCOLL (1-100).	1% NINHYDRIN.	RESULTS.
1 c.c. casein solution	+ 1 drop glycocoll	+ 1 drop ninhydrin and heat	Weak +
1 c.c. " "	+ 2 drops " "	+ 1 " " " "	Fair +
1 c.c. " "	+ 4 " "	+ 1 " " " "	Good +

The above experiment was repeated using a casein poison solution (1-10,000).

TABLE IV.

CASEIN POISON (1-10,000).	GLYCOCOLL (1-100).	1% NINHYDRIN.	RESULTS.
1 c.c. casein solution	+ 1 drop glycocoll	+ 1 drop ninhydrin and heat	+
1 c.c. " "	+ 2 drops " "	+ 1 " " " "	Good +

These experiments indicate that there is something in the casein poison solution which lessens the delicacy of the ninhydrin reaction. Comparison of tables I and II shows that the slight acidity of the casein poison solution is in this respect an important factor, but comparison of tables III and IV indicates that there are other factors and that the diluting of the casein poison solution markedly lessens this inhibitory action so that it is not appreciable in dilutions of 1-10,000.

Some experiments to determine the effect of dilute hydrochloric acid on the ninhydrin reaction.

Experiment I, using Glycocoll (1-1,000) and $n/10$ HCl.

TABLE V.

GLYCOCOLL (1-1,000).	$N/10$ HCl.	1% NINHYDRIN.	RESULTS.
1 c.c. glycocoll		+ 1 drop ninhydrin and heat	+++++*
1 c.c. " "	+ 1 drop $n/10$ HCl	+ 1 " " " "	—
1 c.c. " "	+ 2 drops " "	+ 1 " " " "	—

Experiment II, using $n/50$ HCl.

TABLE VI.

GLYCOCOLL (1-1,000).	$N/50$ HCl.	1% NINHYDRIN.	RESULTS.
1 c.c. glycocoll	—	+ 1 drop ninhydrin and heat	+++++*
1 c.c. " "	+ 1 drop $n/50$ HCl	+ 1 " " " "	+++
1 c.c. " "	+ 2 drops " "	+ 1 " " " "	+
1 c.c. " "	+ 3 " " " "	+ 1 " " " "	—
1 c.c. " "	+ 4 " " " "	+ 1 " " " "	—

Experiment III, using n/100 HCl.

TABLE VII.

GLYCOCOLL (1-1,000).	N/100 HCl.	1% NINHYDRIN.	RESULTS.
1 c.c. glycoll	—	+ 1 drop ninhydrin and heat	+ + + + +
1 c.c. "	+ 1 drop n/100 HCl	+ 1 " " " "	+ + + + +
1 c.c. "	+ 2 drops " "	+ 1 " " " "	+ + + +
1 c.c. "	+ 3 " " "	+ 1 " " " "	+ +
1 c.c. "	+ 4 " " "	+ 1 " " " "	+
1 c.c. "	+ 5 " " "	+ 1 " " " "	—

Small quantities of dilute inorganic acids interfere with and prevent the ninhydrin color reactions.

Experiment IV, using n 10 Oxalic acid.

TABLE VIII.

GLYCOCOLL (1-1,000).	N/10 OXALIC.	1% NINHYDRIN.	RESULTS.
1 c.c. glycoll	—	+ 1 drop ninhydrin and heat	+ + + + +
1 c.c. "	+ 1 drop n/10 oxalic	+ 1 " " " "	+
1 c.c. "	+ 2 drops " "	+ 1 " " " "	—

*++++ indicates a deep blue color.

+ indicates a pale blue or violet color.

Experiment V, using n/50 Oxalic acid.

TABLE IX.

GLYCOCOLL (1-1,000).	N/50 OXALIC ACID.	1% NINHYDRIN.	RESULTS.
1 c.c. glycoll	—	+ 1 drop ninhydrin and heat	+ + + + +
1 c.c. "	+ 1 drop n/50 oxalic	+ 1 " " " "	+ + + +
1 c.c. "	+ 2 drops " "	+ 1 " " " "	+ + +
1 c.c. "	+ 3 " " "	+ 1 " " " "	+ +
1 c.c. "	+ 4 " " "	+ 1 " " " "	+
1 c.c. "	+ 5 " " "	+ 1 " " " "	—

Experiment VI was performed to determine the effect of dilute sodium hydroxide, using n/10 sodium hydroxide.

TABLE X.

GLYCOCOLL (1-1,000).	N/10 NaOH.	1% NINHYDRIN.	RESULTS.
1 c.c. glycoll	—	+ 1 drop ninhydrin and heat	+ + + + +
1 c.c. "	+ 1 drop N/10 NaOH	+ 1 " " " "	+ + + +
1 c.c. "	+ 2 drops " "	+ 1 " " " "	+ + +
1 c.c. "	+ 3 " " "	+ 1 " " " "	+ +
1 c.c. "	+ 4 " " "	+ 1 " " " "	+
1 c.c. "	+ 5 " " "	+ 1 " " " "	+
1 c.c. "	+ 6 " " "	+ 1 " " " "	—

The results of these experiments indicate that N/10 sodium hydroxide interferes with the ninhydrin color reaction to about the same extent as equal amounts of N/50 oxalic acid and as N/100 hydrochloric acid and that small amounts of weak acids and alkalies hinder or prevent this color reaction.

Some experiments were tried to see if sodium chloride interfered with or lessened the delicacy of the reaction.

Experiment VII, using 20% Sodium Chloride.

TABLE XI.

1 c.c. NaCl	+ 1 drop glycoll	+ 1 drop ninhydrin and heat	—
1 c.c. "	+ 2 drops "	+ 1 " " " "	+
1 c.c. "	+ 4 " "	+ 1 " " " "	+
1 c.c. "	+ 6 " "	+ 1 " " " "	+

controlled with water

TABLE XII.

1 c.c. water	+ 1 drop glycoll	+ 1 drop ninhydrin and heat	—
1 c.c. "	+ 2 drops "	+ 1 " " " "	+
1 c.c. "	+ 4 " "	+ 1 " " " "	+++
1 c.c. "	+ 6 " "	+ 1 " " " "	+++++

The above experiment was repeated using one per cent sodium chloride and heating in the oil bath three and one-half minutes instead of two.

TABLE XIII.

1% SODIUM CHLORIDE.	GLYCOCOLL (1-1,000).	1% NINHYDRIN.	RESULTS.
1 c.c. NaCl	+ 2 drops glycoll	+ 1 drop ninhydrin and heat	—
1 c.c. "	+ 4 " "	+ 1 " " " "	+
1 c.c. "	+ 6 " "	+ 1 " " " "	+++
1 c.c. "	+ 8 " "	+ 1 " " " "	++++

controlled with water

TABLE XIV.

1 c.c. water	+ 2 drops glycoll	+ 1 drop ninhydrin and heat	+
1 c.c. "	+ 4 " "	+ 1 " " " "	++
1 c.c. "	+ 6 " "	+ 1 " " " "	++++
1 c.c. "	+ 8 " "	+ 1 " " " "	+++++

This experiment was repeated using 5% Sodium Chloride.

TABLE XV.

5% NaCl.	GLYCOCOLL (1-1,000).	1% NINHYDRIN.	RESULTS.
1 c.c. NaCl	+ 2 drops glycoll	+ 1 drop ninhydrin and heat	—
1 c.c. "	+ 4 " "	+ 1 " " " "	++
1 c.c. "	+ 6 " "	+ 1 " " " "	+++
1 c.c. "	+ 8 " "	+ 1 " " " "	++++

This experiment was repeated using 10% Sodium Chloride.

TABLE XVI.

10% NaCl.	GLYCOCOLL (1-1,000).	1% NINHYDRIN.	RESULTS.
1 c.c. NaCl	+ 2 drops glycoll	+ 1 drop ninhydrin and heat	+
1 c.c. "	+ 4 " "	+ 1 " " " "	+
1 c.c. "	+ 6 " "	+ 1 " " " "	++
1 c.c. "	+ 8 " "	+ 1 " " " "	+++

These experiments indicate that sodium chloride lessens the intensity of the color reaction and gives a lighter and more violet color; and that sodium chloride diminishes slightly the delicacy of the reaction.

We had noticed in some of the preceding experiments that prolonging the time of heating gave much better color reaction and these experiments were per-

formed to determine the effect of a longer heating period. One or two drops of glycocoll (1-1,000) were added to 1 c.c. of water, then ninhydrin was added and heated in the oil bath as before for the time indicated.

TABLE XVII.

	2 MINS.	5 MINS.	7.5 MINS.	10 MINS.	12.5 MINS.	15 MINS.
2 drops glycocoll	—	+	++++	++++	++++	++++
1 drop “	—	—	+	++	+++	++++

Temperature of bath was raised from 120° to 125° C.

1 drop glycocoll	—	+	++	+++	++++	++++
------------------	---	---	----	-----	------	------

These experiments indicate that one gets a much better test by prolonging the time of heating and wherever there is no objection to this, it gives much better results.

Ninhydrin will give a positive reaction with glycocoll in dilution of (1-15,000) when heated two minutes and will give a positive reaction in dilution of (1-45,000) when heated twelve minutes.

Some of the preceding experiments were repeated and the heating in the oil bath continued for twelve minutes instead of two and the temperature kept at 120° C. as in the preceding experiments.

TABLE XVIII.

NINHYDRIN REACTION.	RESULTS (1-100).	RESULTS (1-1,000).	RESULTS (1-10,000).
++			
Casein poison	+++	++++	+
Colon poison	+++	++++	++
Tuberculosis cell poison	++	+++	+
Dog's liver poison	+++	++++	+
Beef's kidney poison	+++	++++	+
Casein	+++++	++++	+
Colon cell substance	+++++	++++	++
Tuberculosis germ substance	+++++	++++	+
Egg albumen	+++++	+++	—
Peptone	+++++	++++	+
Colon residue ⁴	—	—	—
Tuberculosis cell residue	—	—	—
Casein residue	—	—	—

The above suspensions were all carefully neutralized before the experiments. In these experiments the cell substance and the protein poison derived therefrom give positive ninhydrin color test in dilutions of (1-10,000) except egg albumen, which was positive in dilutions of (1-5,000), while the three residues⁴ tested were negative in dilutions of (1-100).

The question naturally arises here; is the ninhydrin reacting substance present in the protein poison or is it cleaved by the heating for twelve minutes?

Early in this investigation, when performing the ninhydrin tests, the solution was heated for two minutes and we got only slight tests with the protein poison in dilutions of (1-100); it was found that keeping the solution either in acid or alkaline solutions at room temperature for two to four days, and then neutralizing and testing gave very much stronger tests, deeper colors. This in-

icates that there is some cleaving of the protein poison and part, at least, of the ninhydrin reaction is due to it.

The toxicity of the protein poison after heating in the oil bath at 120° for twelve minutes was not appreciably diminished.

CONCLUSIONS.

1. Vaughan's portein poisons in dilutions up to (1-10,000) gives the ninhydrin reaction.

2. The proteins from which the poisons are obtained will also give the ninhydrin reactions in dilutions up to (1-10,000).

3. The cell residue in dilutions of (1-100) does not give the ninhydrin reaction.

4. Dilute acids and alkalies interfere with this reaction.

5. Sodium chloride interferes very slightly with the reaction.

6. Prolonging the time of heating makes the reaction very much more delicate.

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⁴Vaughan's Protein-Split Products, p. 165.

THE BULGARIAN BACILLUS IN THE TREATMENT OF VULVOVAGINITIS*

BY MILTON B. COHEN, M.D., CINCINNATI, OHIO.

OF the many problems which have been studied both by clinicians and laboratory men, one of the most interesting is that of combatting infections which have become chronic and in which the parasite seems to have established a resistance to the immunizing substances of the host. One of the methods which has been used to treat such infections is the implantation upon the infected surface of some organism which in cultures produces biologic products capable of destroying the infecting organism or inhibiting its growth. This method of symbiosis has been applied with some success to the treatment of diphtheria carriers by Catlin, Scott and Day¹, and others, and is used more or less as a routine in many hospitals for contagious diseases.

At the Cincinnati General Hospital, a number of investigators have been interested in the subject of vulvovaginitis in children, especially in that form which is usually spoken of as gonorrheal, from which organisms morphologically and culturally indistinguishable from the gonococcus can be isolated. The problem of prophylaxis and treatment of this disease is a very important one as many of the infected children are never completely cured and the cures which are reported are secured only after very energetic and persistent treatment

*From the Pathologic Institute of the Cincinnati General Hospital, and the Department of Pathology of the University of Cincinnati.

with a variety of local injections and irrigations alone or combined with vaccine therapy. In many cases it requires from several months to a year or more to effect a probable cure and frequently after the child is dismissed and treatment is discontinued, the disease recurs. It seems that these vaginitis cases may be included in the carrier group, in which the infecting organisms live and multiply in foci which cannot be reached by the usual methods of treatment. It therefore seemed that it might be possible to implant in the vagina a harmless organism which would thrive in the crypts and folds and so alter the vaginal secretion by its metabolism as to render it no longer suitable for the growth of the gonococcus.

In looking through the literature on the subject, one can find but one reference to such a treatment. Taussig² tried the effect of suppositories of Bulgarian bacilli made from tablets prepared by Parke, Davis & Co. He introduced these into the vagina three times weekly continuing the treatment for from six to eight weeks. Three of his cases improved. There was no attempt made to find out whether or not the Bulgarian bacillus *per se* was responsible for the improvement. Rosenthal³, working in Hayem's laboratory, did a number of symbiotic cultural experiments with this organism and numerous other bacteria among them the meningococcus. He concluded that the metabolic products of the Bulgarian bacillus were very antagonistic to organisms of the meningococcus group, to which the gonococcus belongs.

With this information at hand, it became important to determine whether the Bulgarian bacillus would grow in the human vagina, and whether, when grown there, it would cause the death of the gonococcus or inhibit its growth.

Strains of this organisms were obtained from Parke, Davis & Co., from the Bulgarian Bacillus Products Co., from the stomach contents of a case of gastric carcinoma, and from normal human saliva. After several trials, the following method was adopted as a routine. The centrifuged sediment from a mixed culture of these four strains grown for 48 hours at 37.5° in whey, was mixed with 5 per cent lactose solution. This solution was injected intravaginally twice daily by means of a Luer syringe and a special glass tip which, when inserted through the hymen, plugged the opening and allowed the vagina to be completely filled and the solution to come into contact with its entire surface. During this experiment, all other treatment was discontinued.

Three cases were treated by this method. Each one had a very profuse discharge in which gonococcus-like organisms could be easily demonstrated in smears taken by the swab method. Smears from the vagina were examined twice weekly for the presence of Bulgarian bacilli and gonococcus-like organisms. Not once was the Bulgarian bacillus demonstrated in direct smear even when it was taken within twelve hours after an injection. In two cases, after ten and thirteen days of treatment respectively, the discharge ceased and no gonococci could be found in the vaginal smears. The treatment was discontinued for two days during which time Bulgarian bacilli were demonstrated with some difficulty in cultures from the vagina using the glacial acetic acid method described by Heinemann and Hefferman.⁴ On the morning of the third day a discharge reappeared in which gonococcus-like organisms could be shown. Treatment, using the culture which had been isolated from the vagina two days

previously, was resumed and was continued for three weeks. During this time several negative slides were obtained, but almost always after sufficient search a few typical gonococci could be found. The third case developed measles on the eighth day of treatment and was transferred to the hospital for contagious diseases where the vaginitis was not treated. On the thirteenth day after developing measles, smears made from the vagina were positive for gonococcus-like organisms and negative for Bulgarian bacilli. The bacillus was demonstrated in small numbers by cultural methods.

Although the number of cases treated is very small, and the length of treatment short, it seems that the following conclusions are justified.

1. The *Bacillus bulgaricus* does not thrive in the human vagina and is therefore of little use in the treatment of vulvovaginitis.

2. The results obtained by Taussig in his three cases were probably not due to the Bulgarian bacillus.

I wish to express my sincere thanks and appreciation to Drs. P. G. Wooley, W. B. Wherry and Wade W. Oliver, for their valuable suggestions and assistance.

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LABORATORY METHODS

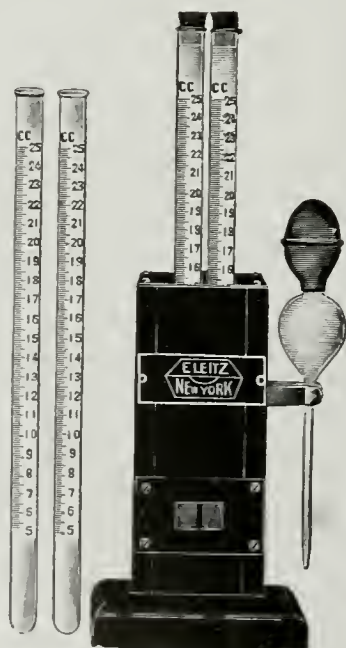
A SIMPLE COLORIMETER FOR CLINICAL PURPOSES*

BY VICTOR C. MYERS, NEW YORK CITY.

ON account of the European war it is very difficult at the present time to secure the excellent colorimeters manufactured by Duboscq and by Hellige. Furthermore, even in normal times these instruments are comparatively expensive on account of their type of construction.

To supply the need for a simple, relatively accurate but inexpensive colorimeter for clinical purposes, the instrument described below has been devised. The instrument is similar in principle to the Sahli hemoglobinometer, but considerably larger. With it one may make such estimations as the excretion of phenolsulphonephthalein in the urine and the uric acid, urea, creatinine and sugar in the blood, etc., with a sufficient degree of accuracy for any clinical purpose.

The instrument illustrated above consists of a box containing two tubes of identical bore (1.2x26 cm.) graduated in tenths from 5 to 25 c.c. The tubes are placed side by side in the instrument, colorimetric comparison being made in the ungraduated portion (0-5 c.c.) through an opening 1x2.2 cm. against a white glass background. To make a colorimetric determination it is simply necessary to dilute the solution in one of the tubes, preferably the unknown, until the depth of color is identical with the other tube. The diluting fluid, generally water, is added with the diluting pipette, inverting the tube after each addition.



*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.

For standards one can best employ solutions of the substance to be determined, i. e., phenolsulphonephthalein, uric acid, nitrogen in form of ammonium sulphate or chloride, creatinine in saturated picric acid solution and glucose in saturated picric acid solution, developing the color in the standard simultaneously with the unknown. Our experience would indicate that for colorimetric work there are no standards quite as satisfactory as those prepared from the substance to be determined and treated in the same way as the unknown.

The calculations with the instrument may be made very simple. If, for example, a blood sugar estimation is being made and the standard glucose solution is the equivalent of 0.1 per cent blood sugar for the conditions used, then a dilution of the unknown to 15 c.c. would represent 0.15 per cent blood sugar, etc.

A POSITIVE TEST FOR BLOOD IN THE STOMACH*

BY MAX B. LEVITON, S.B., M.D., CHICAGO, ILL.

THE test for blood here described is probably more delicate and exact than any hitherto proposed in that the red cells can be seen by direct inspection of the centrifuged fluid under the microscope. It is a modification of Loeper and Binet's¹ test of gastric contents (washings of the empty stomach), except that there is no preliminary lavage and the contents are first neutralized to avoid laking of the blood cells.

The technic is as follows:

The patient is directed to omit anything for breakfast, including liquids, to rinse the mouth and teeth frequently and expectorate any saliva that might form, so that little or none of the cellular elements of the buccal cavity might be swallowed (epithelium, pus cells, blood, etc.). A half glass of water containing a dram of sodium bicarbonate is administered, with a glass of saline solution a few minutes later. The abdomen is then thoroughly massaged and gently squeezed for a few minutes and the contents aspirated by means of a glass irrigating syringe attached to a narrow caliber colon tube (15 French), 4 mm. in diameter. This soft tube produces no trauma as when the ordinary aspirating tube is used and therefore the red cells seen are actually from the stomach. Three small extra holes made in the tube, as kindly suggested to me by Prof. Bird M. Linnell, of Rush Medical College, allows aspiration without a suction action on the mucosa, which is of itself likely to produce bleeding. Precautions should be taken to have connections tight, so that the tube is not swallowed. It is usually necessary to assist the passage of the soft tube down the pharynx by pushing it down with one finger in the mouth to prevent kinking. For this purpose it is better to wear a thick rubber glove to avoid abrasions of the hand on the teeth. The patient assists by swallowing.

All that is necessary is one syringeful, which is centrifuged and examined

*From the Medical Clinic of Rush Medical College, Chicago, Ill.

¹Presse Medicale, Apr. 8, 1914.

microscopically. It is better to wipe the mucus off the end of the tube and expel a few drops before ejecting the fluid from the syringe for examination.

Tests showed that even in concentrated soda solution the red cells remained comparatively unharmed for a long time. The contents that were analyzed up to date almost always showed several red cells, and it is questionable whether under these conditions there are not nearly always a very few erythrocytes in the normal stomach.

The test, therefore, is useful when considered quantitatively, say approximately more than three or four to the high power field. In addition, the other findings suggested by previous writers might be employed, i.e., pus cells, fragments of mucosa, tumor cells, Wolf Junghans' test for dissolved albumin, and lactic acid, besides Oppler-Boas bacilli, yeasts and sarcinæ.

The ordinary coarse tube sometimes brings up blood, especially in the mucus surrounding the opening. As a supplementary test when other findings have proved equivocal, or in sensitive patients who will not take the larger tube this test might prove of some use. Since microscopic bleeding occurs in some cases of gastritis, so-called hemorrhagic erosions, etc., *it is especially valuable as a negative test for blood.*

Special thanks are due to Dr. R. Berghoff, of Central Free Dispensary, Rush Medical College, for many courtesies and the use of cases.

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EDITORIALS

Gastrointestinal Diseases

WHAT we call gastrointestinal diseases are expressions of alterations of function in one or more parts of the gastrointestinal system. They are commonly confused with symptoms, such as hyperacidity, hypoacidity, hypermotility and hypomotility, which are not diseases, but symptoms of disease. Sometimes they are associated with symptoms in other parts of the body than the gastrointestinal tract, and such symptoms call attention to the fact that the various organs of the body are not truly separated or independent, but that they are actually dependent upon one another. So with gastrointestinal upsets the nervous system may be affected, or the urinary system, and it is from these distance effects that we have come to make so much of a group of symptoms, which may be combined into different complexes, in different individuals, which we call, comprehensively, gastrointestinal intoxications or, also, autointoxications.

In order to be clear in our conceptions we should consider first of all the functions of the organs comprising this tract or system.

The function of the stomach and of the intestines also is to contain and retain food for a certain period of time during which the food is chemically changed

by means of ferments. In order to fulfill this function it must be capable of expansion, and, in order to expel the contents at the proper time it must be capable of returning to its original size. In other words, it must be elastic and contractile, and the elasticity and contractility are chiefly the result of the condition of the musculature. The function of the intestine is to carry the food from the stomach to the external world and at the same time to allow time for absorption of food-stuffs into the body. This function depends upon elasticity and contractility and here again the contractility is mainly dependent upon the condition of the musculature. In other words, the function of the gastrointestinal tract, so far as motility (contractility) is concerned, is dependent upon the condition of the muscularis, and so *disease tends to be produced by variation in the elasticity and motility of the various portions of the gastrointestinal tract.*

It is these qualities of elasticity and contractility which prevent stasis in the intestinal tract. Normally the various segments of the tract act in such harmony that the contents are gradually pushed along to their normal destination in a certain normal time. A healthy bowel will not permit of stasis but will carry on its motor functions with ease and regularity. To assist in this function there must be a coordinating or regulating mechanism. This Keith believes he has found in the myenteric (Auerbach's) plexus. He finds that at certain points this plexus is concentrated to form masses (or nodes) which correspond to the nodes in the conducting tissue of the heart, and that at these nodes new rates of peristalsis are inaugurated and persist throughout the segment to which they belong. Evidently *disease of the gastrointestinal tract may occur because of interference with, or damage to, the myenteric coordinating system.*

In order that the food shall be properly prepared for absorption, the necessary kinds and amounts of ferments must be secreted by the glands of the gastrointestinal tract and by the accessory glands, and in order that these shall act properly the contents of the tract must have the proper chemical reaction. In the stomach the reaction must be acid; in the intestine it must be alkaline. Therefore *variations in the quantitative secretion of the various digestive fluids and in the qualitative composition of them, tends to produce disease.*

Gastrointestinal disease arises from abnormal changes in elasticity, motility (contractility), segmental coordination, secretion of ferments, and (chiefly) chemical reaction.

When we consider these four groups we find that we can most usefully combine them into two large groups, for we find that changes in elasticity and motility are apt to occur together; and that changes in production of ferments and the chemical reaction of the juices are apt to be combined. Also we discover that each of these groups are interwoven to such an extent that it is not possible to separate them completely.

The normal course of events in gastric digestion is briefly as follows:

Food enters the stomach and is there kneaded and thoroughly mixed with gastric juice so that the ferments, chiefly pepsin, may act upon the proteins. For the ferment action, a certain degree of acidity is necessary. After a certain time the partially digested food is expelled through the pylorus and enters the duodenum.

What we call gastric indigestion may depend upon a break at any point in

this process, i.e., the muscular movements of the stomach may not be sufficiently active to produce thorough mixture of the food and the gastric juice; the gastric juice may not contain sufficient pepsin to accomplish a normal result; the acid content of the gastric juice may be below or above normal, and then in either case the food may be held too long in the stomach and then undergo abnormal changes which lead to more or less disturbing symptoms or distress.

How shall we account for variations in gastric motility? There is a variation that occurs normally and which depends upon the nature of the ingested food. For instance, carbohydrate foods pass through the stomach most rapidly, proteins are next and fats are slowest. We can say at once then, that a diet very rich in fats will hinder the passage of foods through the stomach. Moreover the ingestion of oils decreases the amount of gastric juice, while ingestion of proteins increases it, and carbohydrates (bread) produce a juice of greatest digestive power. These facts account for the ordinary effects of dietary indiscretions which are commonly transient, but which may persist, if the indiscretions are persisted in, and produce other less transient effects resulting in structural changes.

Now it is quite possible that these normal variations in motility are the result of modification in the acid content of the gastric juice. We know the opening and closing of the pylorus are due to the reaction of the food, i.e., they depend upon the presence of a certain amount of free acid. We know that ingestion of carbohydrates produces an abundant active secretion, and we know that carbohydrates are less active in neutralizing the acid (by chemical combination) than the proteins which also produce an abundant secretion. We would expect then, that the carbohydrates would pass through the stomach more rapidly because they permit the acid to accumulate, and that the proteins would remain longer in the stomach because in combining with the acid they reduce the acidity of the gastric contents. Fats and oils, on the other hand, reduce the quantity of juice and therefore the acid is slow in accumulating.

When the emptying of the stomach is unduly impeded, there is opportunity for fermentation to occur in the contents. We constantly swallow bacteria with our food, but commonly these organisms are rendered harmless because their activities are hindered by the hydrochloric acid. With a decrease of acid they are allowed to act upon the food, and in doing this they produce abnormal acids and gases. The former act upon the mucous membrane producing inflammatory effects; the latter produce distension or "bloating."

Immediately inflammation is produced, an increased amount of mucus is produced, and mucus, being alkaline in reaction, neutralizes more hydrochloric acid, and therefore assists in hindering the passage of food into the intestine. The same stimulation which produces the increase in mucus, irritates the functional cells, so that eventually they become fatigued and produce less secretion. The result of this is that further fermentations are assisted and so (the disease we call it) becomes chronic. When this stage is reached—when inflammation has become chronic, fibrous tissue is produced in the wall of the stomach, the functional cells atrophy and disappear, the mucous membrane becomes thin and gradually becomes less and less able to produce the necessary amount of acid or ferments. When this stage is reached, an incurable condition has been pro-

duced. Before it is reached, there is no incurable stage,—only each more advanced stage is less easily remedied.

But oftentimes it is not so much the relative amounts of carbohydrates, proteins, or fats that is the original cause of indigestion. More frequently it is the quality of the food or other substances that gain entrance to the stomach. Very hot food or drink, very cold drinks, alcoholic beverages, highly spiced foods—all are far more frequent causes. These act by the virtue of their irritative action on the mucous membrane. They produce hypersecretion of mucus and the related stages of fermentation that have already been mentioned, or they cause hypersecretion of gastric juice with subsequent atrophy or hypertrophy of the mucous membrane, resulting on the one hand in absence of secretion or, on the other, in more or less permanent hypersecretion.

What has been said concerning the stomach applies with very few variations to the intestines. Changes in motility, in secretion and absorption depend largely upon factors which are introduced from without, and very commonly are associated with gastric disturbances. Gastric fermentations are prone to lead to intestinal disease, and variations in gastric acidity lead to modifications in the production of pancreatic ferments. In the course of these general modifications which result from gastrointestinal disease then are produced the most various diseased conditions in other organs which cause the symptoms which belong to what we often call the gastrointestinal intoxications.

But all gastrointestinal disease does not depend upon abnormal processes which are primary in the stomach and intestines. Some are the results of changes which are primary in the liver or in the pancreas. As a result of certain changes, quantitative or qualitative variations in the secretions of these organs are produced, and these variations are of far-reaching importance.

It will be remembered that all the above-mentioned juices are poured into the intestine, immediately after the entrance of the acid gastric chyme into the duodenum apparently as a result of the action of the acid. All the intestinal secretions are alkaline. They therefore tend to neutralize the acid of the gastric juice and therefore they produce the conditions which are necessary for opening of the pylorus. So long as the duodenal contents are acid, the pylorus remains closed. *An insufficient amount of alkaline juice from the digestive glands tends to produce disease.*

The activity of the pancreatic juice depends upon the activation of its proteolytic pro-enzymes by enterokinase which exists in the succus entericus, which is caused to flow by the presence of pancreatic juice in the intestines. *Absence of enterokinase tends to produce disease.*

The flow of all the intestinal juices, pancreatic, bile and succus entericus, is brought about by the action of the secretion (produced in the cells of the intestines by the action of the acid gastric chyme) upon the cells of the pancreas, liver, and intestinal glands. *Absence of sufficient acid in the gastric juice tends to produce lack of intestinal secretion, and hence to produce intestinal disease.*

If the pancreatic juice is not activated, proteolytic digestion suffers. If the bile is not present in sufficient amounts, the utilization of the fats of the food is reduced or hindered. The conditions produced in this way are ones that result from insufficient preparation and absorption of food. The insufficient preparation allows of increased bacterial activity and tends to the production of abnor-

mal substances, or substances in abnormal amounts, which, being absorbed, may give rise to intoxications, providing they are not neutralized.

But aside from these there are changes which occur in the glandular organs themselves which produce similar effects. Diseases of the liver, of the pancreas, of the wall of the intestines, produce the same effects. In this group we have the fibroses of the liver and the bile passages which decrease the flow of bile and produce qualitative changes in it. Inflammation of the pancreas or its ducts produces quantitative and qualitative effects upon the pancreatic juice and similar changes in the wall of the intestines tend to reduce the formation of enterokinase and secretin.

Just as gastrointestinal disease may have its starting point outside that tract, in the accessory glands in the abdominal cavity, so also it may have its origin in the mouth. The mouth is the most important portal of infection of the body, and is perhaps more prone, especially in the years after the first decennium, to infection than any other part of the body. Moreover diseases of the mouth, especially those in which the teeth are involved, are every day assuming greater importance in medicine. It is becoming more and more evident that the lesions of many accepted clinical complexes are secondary to primary infections of the oral cavity and also that many obscure symptoms are the sequels of chronic focal infections which in very many cases are located in the teeth or in their surrounding tissues. Hence it is that pyorrhea alveolaris and peridental and dental abscesses are becoming of as great importance to the physician as to the dentist, and that there is every reason to believe that, in certain directions at least, the practice of dentistry will have to be revolutionized even as medical views concerning the importance of the mouth as a source of generalized disease are being changed. It is coming to be realized that foci of infection in or about the mouth may be the starting points of such so-called diseases as rheumatism, endocarditis, appendicitis, gall-bladder disease, gastric ulcer, osteomyelitis, hay fever, asthma, urticaria, and even certain goiters. When we say in or about the mouth we have reference to the tonsils, the nasal sinuses and antra, as well as the teeth and peridental tissues.

The reason why the mouth is so apt to be a primary source of trouble is that it, more than almost any other part of the body, is opposed to the action of external injuries, and that modern habits of eating and drinking produce conditions which, without proper precautions, lay the foundations for various sorts of local and general trouble. Too hot or too cold foods taken into the mouth do two things;—they injure the epithelial surfaces and so predispose to infection, and they tend to damage the teeth and make them more easily attacked by bacteria. Food which is too carefully chosen for tenderness and easy mastication, or which is finely divided, tends to collect between the teeth or along the margins of the gums, and there ferment. Thereupon inflammations or caries, or both, result. The more antique habit of not eating hash but of exercising the teeth and gums on the food made the toothbrush less essential for health than it now is. Cattle which are fed on soft food,—as, for instance, still slops,—almost exclusively, have dental trouble that animals under normal circumstances never have. It is exercise which keeps the teeth and gums in good condition and if this cannot be accomplished by means of the food, some other agent is required and this is usually the toothbrush assisted too frequently by the dentist's drill. Den-

tal floss takes the place of the older meat fibers which stuck between the teeth and had to be picked out after the prandial exercises (or even sometimes during them). In this process the spaces between the teeth were cleaned.

From the standpoint of dentistry it is important to remember that crowns and bridge-work, as they are too commonly applied, while they may be something of a convenience, are nevertheless a very real danger in producing focal and general infections and intoxications. To the truth of this x-ray pictures of the teeth will testify. Any apparatus which tends to produce mechanical irritation is a dangerous one, for while it is true that foreign bodies may produce little or no damage provided they exist in aseptic surroundings, yet in the presence of infective organisms they act invariably to intensify the reaction between tissue and germ. A bridge which offers opportunity for the collection of food and bacteria about it; a crown or a bridge which is attached to or which covers a tooth whose nerve has been killed (by which procedure the tooth itself has been made a foreign body), especially if complete sterilization has not been carried out, is a menace to the general health of the patient, who had far better have fewer teeth than such an appliance. A little macerated pulp at the bottom of root canal and one living bacterium buried at the bottom of a root canal under an impervious cap or filling, is not to be preferred to a vacant space in the tooth line. It is under such conditions that root and alveolar abscesses develop and remain for long periods of time affecting the health of the individual by producing bacteria and toxins to enter the blood stream by which they are carried to the heart, central nervous system, muscles, bones, or other tissues and organs.

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—P. G. W.

Death by Strangulation

MEDICO-LEGALLY it is desirable to distinguish between hanging and strangulation, because most of the cases of hanging which the expert is called upon to investigate are suicidal; while death from strangulation is generally homicidal. Strangulation is defined by Tardieu as "an act of violence

in which constriction is applied directly to the neck, either around it or in the fore part, so as to prevent the passage of air and thereby suddenly suspending respiration and life." As has been pointed out by Taylor, this definition includes hanging, but as the same author goes on to say, "while there is only one method of producing death by hanging, there are various methods of producing death from strangulation. A person may be strangled by the use of a cord, band, or ligature drawn tightly around the neck, or by manual violence to the front of the neck, whereby respiration is prevented."

Suicidal strangulation is possible, but is exceedingly rare. The question has been asked many times whether or not it would be possible for one to compress his own windpipe to the extent of inducing death. On the negative of this question it is argued that as soon as the period of unconsciousness is reached,—possibly before this time—the fingers will lose their power of compression, air will again pass through the larynx, and death will not result. This argument is probably correct, but it would be possible for a person to pass a cord a number of times around his neck and then by pulling on both ends kill himself, because, on account of the number of coils, the pressure would not be relieved when the hands ceased to pull. Indeed, cases of suicide by strangulation in this way have been reported. Another method of suiciding by strangulation is to pass a cord around the neck, then put a stick of some kind between the cord and the neck, and twist the stick. Several cases of suicide by this method have been recorded. Possibly there are other maneuvers which might be resorted to with success in the accomplishment of this purpose. However, unless there be some direct and positive evidence that the act has been self-committed, it is to be presumed that death from strangulation is homicidal. It is very easy for a strong man to so compress the larynx of a child or a woman with his fingers as to prevent any cry for help, and to induce death within a few minutes. In fact this method which has been resorted to for the purpose of robbery and murder, is so well known that a special name has been given to it, and it is designated as the crime of "garroting" on account of its resemblance to the Spanish method of executing criminals. The garrot consists of a brass collar, which is placed about the neck and gradually tightened by a screw behind. This screw has a sharp point and it not only tightens the collar but pierces the spine.

When death has been caused by strangulation with the hands there are usually external marks to indicate the nature of the injury. In fact the murderer who employs this method generally uses more violence than is necessary. He frequently tears the flesh with his nails, bruises the soft tissue, and in many instances crushes the cartilaginous structure of the larynx. In some instances the injury thus done is so great that it is quite evident that it could not be self-inflicted. Frequently the hyoid bone is fractured. In a case of this kind Schüppel proved that death had been thus caused in the body of a child aged ten who was found in the debris of a burned building.

Homicidal strangulation may be accomplished by the powerful hand of the murderer or by means of a cord or rope. When something is placed about the neck, and death is induced by tightening or twisting this, the victim usually struggles sufficiently to produce evidences of violence. When the cord is small

it may cut the neck deeply, and indeed this may be accomplished when a handkerchief is used for strangulation by bringing the constriction to bear solely upon one hem or by twisting the handkerchief and thus converting it into a cord. In some rare instances where death has been induced by strangulation, the murderer has attempted to make it appear that the injury has been self-inflicted, but usually these attempts overstep themselves, and aid in detecting the crime. Sometimes the medical expert is asked whether death was caused by strangulation or induced in some other way, and the injury to the neck inflicted subsequently. This question is not always easy to answer. So far as external appearances are concerned, the presence of ecchymoses on the neck, protrusion of the eyeballs and of the tongue, together with a livid, swollen appearance of the face, constitute marked indications of death from strangulation. However, Caspar has pointed out that a ligature applied about the neck within an hour or two after death may cause ecchymoses which cannot be distinguished from those produced during life, and there may be protrusion of the eyes, lividity and swelling of the face and extrusion of the tongue in death from other causes. So far as evidence furnished by postmortem examination is concerned, it may be stated that this may be of the greatest value. As a rule the lungs are congested and some of the more superficial of the air cells may be ruptured, leading to a more or less extensive emphysema, and giving to the surface of the lungs a mottled appearance with patches covered with thin white layers of tissue from which when punctured air escapes. While this is the usual condition after death from strangulation, it is not constant. When present and accompanied by evidences of external violence applied to the neck, the proof of death by strangulation is positive, but when absent we cannot say that death has not been caused by strangulation. Moreover postmortem examinations in these cases are frequently not made until after many days, and putrefactive changes may be such as to confuse the most experienced expert. After death from strangulation the heart presents no constant or uniform condition, although it generally contains fluid blood, and the right side is more prominently distended than the left. The brain is congested, as it is in most all cases of death from asphyxia.

When the expert is called upon to examine a body supposed to have been strangled he must make most careful examination, not only of the neck, but of every part of the surface for evidences of external violence. He should give attention to the limbs, especially the legs, wrists and arms. The murderer is likely to hold the hands of his victim, or to throw him by striking his feet from under him. In garroting, the murderer frequently presses upon the chest of his victim, sometimes upon the abdomen. It not infrequently happens that on account of such pressure vomiting results, and after death vomited matters may be found in the pharynx, and even in the larynx. In case the victim is a female, evidences of injury to the sexual organs should be sought for and any other indications of rape should not be overlooked. If wounds be found the question arises as to whether it would be possible for them to be accidental or self-inflicted. In many cases of death from strangulation it is well to examine the stomach for poisons, especially those of narcotic action, inasmuch as the homicide sometimes resorts to these for the purpose of rendering his victim help-

less. The presence of a cord or rope about the neck should not always be considered proof that death has resulted from either strangulation or hanging. There are several cases upon record in which the murderer after accomplishing his purpose in some other way has placed a rope or a cord about the neck of his victim in order to make it appear that death by hanging had been self-inflicted.

Strangulation With a Cord.—The word cord is used here, to mean anything that is long enough and pliable enough to be twisted about the neck. The things used in strangulation are almost as diverse, probably not quite so, as in hanging. There is this fundamental difference between hanging and cord strangulation, in the former the force that compresses the neck and causes death is the weight of the body, fully or only partially applied, while in the latter the weight of the body is in no way concerned and the active force is that used in tightening the cord about the neck and is exerted through the hands of the suicide or the murderer as the case may be. As a rule, in hanging the cord is placed directly under the chin, above the larynx or over its upper portion and between the thyroid cartilage and the hyoid bone. In strangulation the cord is usually placed lower down, just above, immediately across or just below the thyroid. In hanging, with one exception, the lowest part of the cord is in front, and in each side it passes backward and upward. The exception is when one hangs himself in a horizontal position, especially with his face downward. In strangulation the cord lies nearly or quite horizontally. In hanging the passage of air is prevented by the base of the tongue being carried back against the posterior wall of the pharynx, while the posterior nares are tamponed by the soft palate and the apex of the larynx is carried upward and backward. In strangulation the tongue and soft palate may not be materially affected in position. The exclusion of air is more speedily and completely secured in hanging than in suffocation. In hanging the interruption of the flow of blood is complete; the jugular veins, the carotid and vertebral arteries are all occluded. In strangulation the veins are soon occluded, the carotids slowly and often only partially, while the vertebral arteries are never closed. The insult to the vagus center through the superior laryngeal may be the same in hanging and in cord strangulation. The phenomena of death are much the same in the two, but they proceed more slowly in strangulation, the stage of dyspnea is prolonged, consciousness is not so quickly lost and death is not so sudden. When the cord is placed across the thyroid, much force is necessary and in the majority of instances the arrest of the air stream is not complete. The vertebral arteries still carry some oxygenated blood into the brain and consequently the arrest of brain function is not so abrupt as it is in hanging. With the flow of blood into the brain only partially arrested while the return is wholly stopped, engorgement of this organ necessarily follows. It must not be inferred from the above that death cannot be speedily induced by strangulation, and it is only by comparison that the death agony can be said to be prolonged. In death by hanging the loss of consciousness is instantaneous, in the more violent forms of strangulation it may be measured in seconds, but, under ordinary conditions, in minutes.

The postmortem appearances after death from cord strangulation usually differ quite markedly from those observed in hanging. Externally there is venous

engorgement of the scalp and face, and, in short, of every part above the cord. The cord grooves or marks may be one or more, depending upon the number of times the cord has been brought about the neck. These may be narrow and deep or broad and superficial depending upon the nature of the cord and the degree of force applied. As has been stated the cord grooves lie lower than in hanging and pass around the neck horizontally. Usually the track of the cord can be traced wholly around the neck, over the back as well as along the throat. Since the body is not suspended hypostatic ecchymoses so prominently seen on the dependent portions of the suspended corpse are lacking or are to be found on the back if the corpse has been for some hours in a supine position.

According to Reuter¹ there are two groups of marked blood extravasations quite generally present after death from cord strangulation. The first is in the neighborhood of the strangulation groove, generally above it, and consists of hemorrhages in the cervical muscles, their sheaths, the intermuscular tissue, the capsule of the thyroid and under the perichondrium of the laryngeal cartilages. These are supposed to be due to the direct pressure of the cord. The second group is quite removed from the line of the cord and is distributed at points of greatest congestion, as near the angle of the lower jaw, in the floor of the mouth, in the tonsils, and in the connective tissue behind the trachea. Fracture of the laryngeal hyoid framework is not frequent, but one may find fracture of the thyroid and annular cartilages, less frequently of the arch of the hyoid bone. Seldom hemorrhage into the sheaths of the blood vessels is found and rupture of the intima has been reported only once (Ziemke).

On dissection, congestion is prominent, especially in the brain and soft parts of the skull; moreover, the mucous membrane of the pharynx, the base of the tongue and the esophagus is congested and often shows numerous ecchymoses. Sometimes the surface of the trachea is covered with a white or blood-stained foam that results from a pulmonary edema due to the prolonged dyspnea.

It seems to be generally believed that suicide by strangulation with a cord is a difficult feat, and it is true that the majority of deaths from this cause are not self-inflicted. However, the number of suicides in this manner, while less than the number of murders, makes up from 20 to 30 per cent of the total deaths from this cause, and possibly if the strangulation of the newly born be excepted, the percentage of suicides is larger. The courage and resolution displayed by the suicide in this direction are quite remarkable, even the use of a stick to make the pressure greater by twisting the cord has been resorted to not only in murder but in suicide. A case is reported in which a woman used her own hair to strangle herself. It will be seen that the question between suicide and murder is not always an easy one. The strangulation of young children in this manner is either homicidal or accidental, generally the former. In adults it may be suicide or murder, more frequently the latter. In twenty-two cases reported by Reuter, seven were suicide. The kind of knot has in two instances at least given a clue to the hand that tied it. In one of them Tardieu recognized the knot as one used by artillerymen and this led to the arrest of the right man; in another the knot was that employed in a factory where shawls were made and this enabled Hofmann to show that it was suicide. In murder the vio-

lence of the act is generally greater and death more speedy. On account of the greater violence more local injury is done and this shows itself in more frequent fractures. On account of the more speedy death the cyanosis of the face and the congestion of the scalp, soft parts of the skull and the brain is less marked in murder than in suicide. In murder, especially when the victim is an adult, other injuries are often done, such as those that result from blows on the head and stab wounds of the chest. However caution must be exercised here for the desperate man intent on suicide may wound and bruise himself severely before completing his task by strangulation. The marks upon children when killed by strangulation are often slight compared with those seen in adults. This follows from the helplessness of the victim, the feeble efforts made to free itself, the softness of the tissue, the use of a broad cord, and especially to the fact that suffocation is often combined with strangulation.

Accidental death by cord strangulation may happen and some interesting cases have been reported. Smith tells of a boy who was accustomed to carry a weight suspended about his neck. One day he was found dead in his chair; the weight had fallen pulling the cord tight about his throat. Taylor reports the case of a girl who was carrying a heavy basket suspended from her shoulders to market. She climbed onto a wall and sat down to rest, the basket fell behind her pulling the cord about her throat and strangling her to death.

The differentiation between hanging and cord strangulation is usually easily made by the direction of the cord groove, as has already been pointed out, but when one hangs himself while lying on his face, in which case the lines will be horizontal and if the rope has been twice passed about the neck the groove may extend all the way around.

Reuter formulates the following as typical evidences of death by cord strangulation:

1. Cyanosis of the face with or without ecchymoses.
2. Numerous ecchymoses on the lids and conjunctiva.
3. Congestion of the scalp, brain and the meninges.
4. Hemorrhages in the soft tissues of the throat, which lie either near or above the strangulation grooves and in the sheaths of the cervical muscles, in the intermuscular connective tissue, and under the perichondrium of the laryngeal cartilages, or removed from the strangulation groove and near the angle of the lower jaw, in the floor of the mouth, in the tonsils and in the mucous membrane of the pharynx.
5. Marked injection of the mucous membrane of the upper air passages together with numerous small ecchymoses.

Strangulation with the Hand.—When the larynx is seized laterally between the thumb and fingers, as has been shown by the investigations of Langreuter,² it is easily compressed, much more readily than when the pressure is directly backwards. This lateral pressure is the most efficient agent in the causation of death by strangulation with the hand. In fact, however, the pressure is generally not exclusively from the sides but at the same time upwards and backwards. Theoretically the ends of the fingers and thumb are in position in this act to compress the large vessels of the neck, but actually this seldom occurs, but it does occur in exceptional instances. Ziemke states that repeatedly in this

form of strangulation in the newly born he has found hemorrhages in the vascular sheaths and in one case he observed rupture of the intima of the right carotid identical with that much more frequently seen in death from hanging. Irritation of the superior laryngeal nerve is more pronounced in this form of violence than in either hanging or in strangulation with a cord. Animal experiments have shown that mechanical irritation of this nerve may cause sudden arrest of either the heart or the respiratory movements. Hofmann observed like effects on compressing with his fingers the larynx of a tracheotomized dog. Indeed, he found that a single short compression of the larynx sufficed to temporarily arrest respiration and that continued pressure caused dyspnea to follow the temporary arrest. Indeed pressure or other injury to the larynx may cause death through the nerves without closure of the windpipe. Brouardel tells of a girl being killed by a light blow on the larynx and he speaks of this as an instance of laryngeal shock. However, death is seldom due to nervous insult only and fracture of the laryngeal cartilages occurs in most instances of death from strangulation with the hand. It has been claimed that death has resulted from merely grasping a man by the larynx but in these instances it is more than probable that the force of the grasp has been underestimated. Fracture of the thyroid is not uncommon and it seems that in the aged this cartilage may become exceedingly brittle. The mechanism of death by strangulation involves in most cases more or less marked injury to the larynx or thyroid with the effect through the nerve and in rare instances compression of the blood vessels. In lateral compression death may be wholly due to occlusion of the air passage.

After death from this form of strangulation the face is cyanotic; there are ecchymoses in the skin and especially in the eyelids and the conjunctiva; marked congestion of the scalp, soft parts of the skull, the brain and the meninges; there are also ecchymoses in the mucous membrane of the air passages and in the viscera.

The most characteristic external effects of this manner of death are seen in the marks left by the finger nails on the throat. As a rule these marks are more numerous on the left side, because on this side are placed the fingers of the assailant provided he be right handed. It must not be supposed that the thumb makes only one mark, or that the fingers always make four and no more. As the assailant tightens his grasp and his thumb and fingers press the soft parts in, the marks made upon either or both sides may be numerous. Moreover, when the victim is strong enough to make any decided resistance there may be scratches over the face and about the throat quite removed from the larynx. Rarely there are marks in those who have met death by hanging or by cord strangulation that may have the appearance of scratches caused by the finger nails. Liman reports a case of this kind in one hanged and on investigation he found that they were due to small buttons on the cord that had been used by the suicide. Scratches with the fingers are not likely to lie in the same plane but are unevenly distributed. The finger marks may be wholly wanting when some article of clothing lies between the hand of the assailant and the throat of the victim and the story is told of a villain who had repeatedly practiced this form of murder and that he always did it with a gloved hand. Brou-

ardel³ reports a death from choking, in which attempt the skin showed no marks. There were extensive hemorrhages in the deeper tissues, and two similar cases have been observed by Messerer.⁴

The hemorrhages found on dissecting the throat of one murdered in this way are more extensive than those after either hanging or cord strangulation. They usually lie in and between the muscles, in the tissues of the thyroid and in some cases in the submaxillary salivary glands. In nineteen cases Reuter found hemorrhages, in eleven in the cervical muscles, and especially in the tissue of the thyroid, four times in the tonsils and pharynx, and seven times in the larynx. Laceration of the muscles has not been reported, but, as has been stated, fracture of the cartilages is frequent. The fractures, however, are more common in the aged, and in the newly born they may not, and often do not, exist. Fracture of the hyoid bone is rare and the trachea is not often injured. Patenko⁵ has studied the mechanism of fractures of the larynx in choking and concludes that in some persons ossification of these cartilages begins as early as 35 years of age and may progress to such an extent that fracture is relatively easy. It must not be inferred that fracture of the larynx in the young may not be caused by choking and such a case has been reported by Stolfer⁶ in a youth of 15.

With the exception of the unique case reported by Binner⁷ there is no known instance of suicide by strangulation with the hands. When the larynx is compressed by one's own hand, the hand loses its grip before death is reached. The case reported by Binner is interesting but is doubtful. A woman who was known to have tried to choke herself was found dead in a squatting posture by the side of her bed. Her elbows rested on her knees and pressed against the side of the bed. Her hands formed a fork in which rested her throat while her head hung forward and downward.

This form of strangulation being always homicidal is often combined with other injuries to the body. The assailant knocks his victim down or pushes him over, then with his knee on the chest he proceeds to strangle him. The pressure on the chest may cause a bruise or an abrasion and not infrequently fracture of one or more ribs. Again, the assailant wounds his victim by a blow on the head or a stab in the chest and then strangles him while lying in a helpless state. In some instances strangulation with the hand is completed with a cord, or death having been caused by the former the murderer may fasten a cord about the neck of his victim to make it appear that he has suicided. The differential diagnosis between strangulation by the hand and by the cord is easy when the external marks are distinct and distinctive, otherwise quite difficult. Reuter is of the opinion that the more profuse extravasations of blood after death by the hand and their locations, especially in the tissue of the thyroid, the submaxillary glands and the tonsils, are sufficiently characteristic to justify a diagnosis. They certainly are if the external markings are confirmatory, otherwise they are not. There are several reported cases in which the murderer has suspended the corpse of his victim in order to awaken the suspicion of suicide, but fortunately the differential diagnosis between hanging and strangulation by the hand is generally quite easy and certain. In the first place a comparison of the external marks about the throat is usually sufficient, but these

failing, the differences found on dissection of the throat are likely to be satisfactory. Fracture of the annular cartilages and of the thyroid and large blood extravasations in the soft parts of the throat are very rare in death by hanging, and indeed, occur only with a long drop, while they are practically common and certainly are typical in strangulation by the hand. Strassmann reports a case of a woman found hanging in an open sling of soft cloth. The external throat marks were not distinctive, but dissection showed double fracture of the annular cartilages, fracture of the superior bone of the thyroid and four hemorrhages in the mucous membrane of the larynx. After these findings he did not hesitate to testify that death had been induced by strangulation with the hand and not by hanging. Wilhelmi⁸ was able from the presence of fractures and their nature to show that a woman who had been found suspended had actually been strangled by hand, and in this instance the body was not examined until it had been in the grave for five and one-half weeks.

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—V. C. V.

The Nephroses

THE term nephrosis is an inclusive one. It includes all the diseases (except tumors) from which the kidneys suffer, from mere edema to the true inflammations, whether suppurative or nonsuppurative. It therefore includes what we speak of as nephritis, or Bright's disease.

The term nephritis includes two conceptions: one of them clinical, the other histological, or anatomic. Clinically it means the presence of certain qualitative or quantitative urinary variations, which are, generally speaking, increase or decrease of the total amount of urine and the presence of albumen and casts in the urine. In the anatomic sense it means this and something more. It means inflammation, which is to say the kidney is the seat of certain vascular and perivascular changes which we associate with a very distinct histologic picture, in which vascular dilatation, congestion, and exudation are in the foreground. Albumen and casts in the urine may be produced by any process which disturbs sufficiently the nutrition (or metabolism) of the renal cells. Inflammation is one such process. Mere local acidosis, which produces edema without the signs of inflammation is another.

There are three main types of renal lesions, the simple nephroses, including simple edema and simple necrosis; the inflammatory nephroses or nephritides; and the disuse nephroses, including the arteriosclerotic and renal atrophies.

THE SIMPLE NEPHROSES.

This group is one which is fundamentally the most important. In it are included all those types of renal disturbance which are the result of partial or

complete lack of oxygen, from the edema of the athlete, and that of the soldier on the march, both of which are benign and transient, to the sudden complete necrosis of infarction. In it belong the so-called "alterative nephritides" of certain texts, and the toxic necrotic conditions which are the result of the action of organic or inorganic poisons. In all these albumen and casts appear in the urine; in all the quantity of urine is diminished. The main difference that needs mention in discussing these simple nephroses, is that in the kidney of edema, the organ may return to a completely normal condition very rapidly; in the toxic degenerated and in the infarcted kidney the chances are that this does not occur, but that an increase of scar tissue is formed as a part of a secondary inflammatory process. In the case of infarcts the scarring is localized; in cases of certain chemical intoxications it is diffuse.

It may be interesting in connection with these simple nephroses to recall Osler's remark, that "every case in which albumen is present must not be called acute Bright's disease, not even if tube-casts be present. Thus the common febrile albumenuria, although it represents the first link in the chain of events leading to acute Bright's disease, should not be placed in the same category."

THE ATROPHIES.

Similar in some respects to the simple nephroses are the atrophies. These, like the former, depend for their urinary phenomena upon lack of oxygen, but the cause, in this instance, lies not in the composition of the blood, but in the condition of the blood vessels, which have been so changed by one or another factor, that the blood, qualitatively perfect though it be, does not reach the cells in sufficient amounts to keep them at a physiologically active level. What happens to the kidney in arteriosclerosis of a simple type, is exactly what would happen to the kidney of a moderate simple edema, provided the edema were chronic, which is to say, provided the circulatory conditions remained the same. In the renal edema of the sprinter the oxygen supply in the blood is not quite sufficient for the cells of the kidney. In the arteriosclerotic kidney, the oxygen in the blood is sufficient but it cannot reach the cells rapidly enough. The result of this decreased access of the cells to oxygen is that they undergo atrophy, become smaller, and some of them disappear, or, if the progress of the vascular disease is somewhat more rapid, they undergo degeneration and disappear, and in vanishing leave the stroma of the organ apparently more abundant, relatively increased, and this tends to produce the appearance of sclerosis or fibrosis in the organ. Actually such an atrophic organ contains no more fibrous tissue than a normal organ,—it merely possesses less parenchyma.

THE NEPHRITIDES.

This group of the nephroses is characterized, so far as the renal parenchyma is concerned, by all the anatomic changes which are present in the former groups, and by certain other features, which are clearly inflammatory. In all the types one phenomenon dominates the picture, and that is the one we term exudation which may or may not be associated with fibrosis. It is from the obviousness of the associated fibrosis that we are led to speak of an acute or

a chronic inflammation. Fibrosis in the absence of exudation does not spell inflammation though it may be a sequel of it. A sclerotic kidney is not necessarily one of interstitial nephritis.

It has been customary for many years, and will undoubtedly remain customary for a very long time, to speak of parenchymatous and interstitial nephritis, by which is meant types of inflammation which affect the parenchyma of the kidney, or the intestinal tissues as the case may be, predominantly. One goes further if he follows the texts and divides the nephritides into other types, such as simple, hemorrhagic, and suppurative; and as glomerular, tubal, and diffuse; and further, as acute and chronic. These terms are, however, merely helpful in characterizing nephritides from the standpoint of general extent. The process in all the cases is the same. It varies only in the extent to which it affects the kidney, in the type of exudation, and the rapidity of its development. Moreover the inflammatory process always starts in the neighborhood of the blood vessels, and therefore it happens that at but one place in the kidney can an inflammation be spoken of as being primarily parenchymatous,—that place is the glomerulus. All other forms of inflammation are essentially interstitial in type, and the peculiar and sometimes characteristic changes which one observes in the parenchyma are secondary ones due to disturbances of metabolism resulting from poisons which reach the cells from foci of inflammation, or from lack of oxygen which is the result of perivascular conditions. There are times when these secondary changes dominate the picture; when the parenchyma has undergone fatty or soapy degeneration, as, for example, in the “large white kidney.” Under such circumstances we have been in the habit of speaking of a chronic parenchymatous nephritis, a designation which we may preserve if we wish, provided we realize that it is not that, but a type of interstitial nephritis with abundant secondary degeneration—a *diffuse nephritis*. This is very reasonable, because if we study the other classical type of kidney, the “small red” one we find similar changes, though not so evident. In this the focal inflammatory fibrosis dominates the picture, and obscures the parenchymatous changes. Perhaps it were best to say that in all nephritides parenchyma and interstitial tissues are both affected, but that in one the degenerative changes predominate; in the other the productive. Perhaps it is best to speak of *diffuse* and *focal nephroses*. All of these remarks apply equally to the suppurative and tuberculous kidneys. In these the only difference is that the lesions tend to be more obviously and completely focal than even in the interstitial form, and therefore they acquire specific characteristics.

This would all be very simple were the forms of nephroses simple, which, as a rule, they are not. As a rule they are combined, at least this is apt to be almost completely true except in cases in which death has been produced suddenly by trauma. That the conditions existing in the kidneys may undergo sudden changes is shown in many clinical cases. For instance, a person who has a well developed interstitial type of nephritis does very well for long periods of time. The vascular and perivascular changes have advanced to such a stage that the compensatory powers of the organ are quite limited. Much of the parenchyma has disappeared, but the portion which remains is physiologically active and produces a normal amount of fluid, with little or no albumin and

few or no casts. If at such a time he meets a sudden traumatic death, the kidney would appear as a "small red kidney," a primarily contracted kidney. But let him suffer an attack of enteritis; let him absorb a few more colon bacilli; a too large quantity of a poison. He dies in uremia (cerebral edema) and the kidney at postmortem is apt to be gray and then we are apt to say that it is a "secondarily contracted kidney," or small gray kidney. And it is quite probable that under such conditions unless he makes use of the clinical details the pathologist may insist that what he finds tells the whole story.

It would seem then that for the sake of simplicity the following classification would be serviceable:

- A. The diffuse nephroses—(acute intoxication).
 - Simple edema, and necrosis.
 - Acute diffuse nephritis (acute Bright's).
 - Glomerular nephritis.
 - Hemorrhagic nephritis.
 - Chronic passive congestion.
 - Chronic diffuse nephritis (chronic parenchymatous nephritis).
 - Glomerular nephritis.
- B. The focal nephroses—(vascular or infective).
 - Infarction.
 - Arteriosclerosis.
 - Acute interstitial (septic or simple) nephritis.
 - Chronic interstitial nephritis.
- C. Combination of focal and diffuse forms.

Such a classification has one value if no other; namely, that it allows of a better understanding of the urinary findings,—that is to say, it permits the clinician and the pathologist to stand a little closer together.

URINARY CHANGES IN THE NEPHROSES.

- I. Diffuse nephroses—(acute and chronic).
 - Decrease of urine.
 - Casts present.
 - Albumen in very noticeable amounts.
- II. Focal nephroses—
 - a. Acute—
 - Urine may be decreased, depending upon the number and extent of the lesions.
 - Casts present.
 - Albumen in moderate amounts.
 - b. Chronic—
 - Little or no decrease of urine.
 - Few or no casts—these come from the diseased foci.
 - Little or no albumen.
- III. Combination Forms—The urinary changes vary.

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—P. G. W.

Diabetic Therapy

IT has been a usual comment of those who have watched the development of scientific knowledge in this country, that it is particularly in the application by practical methods of principles discovered elsewhere that the American investigator excels. In certain of the medical sciences this comment is particularly applicable, for it has been by developing in a practical way the fundamental discoveries of European investigators that the American surgeon has acquired so world-wide a fame. The development of surgical technic has been going on for many years, but it is only recently that a similar advancement can readily be recognized to be under way in the domain of internal medicine, and in no branch of this is it more conspicuous than in the therapy of diabetes. It is now many years ago since Bouchardat, Naunyn and Cantani first suggested that severely diabetic patients should be starved until the urine became, if not entirely, yet very nearly sugar-free, but the suggestion was not generally adopted, because it had meanwhile come to be clearly recognized that the coma to which many such patients succumb is in some way related to the appearance in the urine of the so-called acetone, or acidosis, bodies, and that the amount of these often increases alarmingly before the starvation had got well under way. The starvation method of treatment had not been developed sufficiently, nor had its beneficial influence on the course and outcome of the disease been clearly enough demonstrated to make the majority of physicians feel justified in adopting it to any great extent. Acting partly on the suggestions offered by the experience of European clinicians, and partly because of results obtained by observing animals rendered more or less diabetic by partial removal of the pancreas, Allen undertook the thorough trial of starvation in specially selected cases of diabetes kept under constant observation in the wards of the Rockefeller Hospital. He showed among other things that in the great majority of even severe cases the acidosis which may develop during the first few days of starvation afterwards subsides, so that in a surprisingly short period of time the urine contains neither sugar nor more than small quantities of the acidosis (acetone) bodies. Sometimes, however, the treatment is not so successful. When the ability of the blood to combine, without disturbance of its reserve alkalinity, with all of the acids produced by the faulty

condition of metabolism—of which the appearance of acetone bodies in the urine is an indication—is studied in diabetic cases, a thoroughly reliable criterion is furnished of the effect of the treatment.

The technic of several of the laboratory methods employed in securing these data has already been described in this journal, and for the present we would direct attention to a recent communication by Woodyatt in which the general principles underlying the development of acidosis are admirably set forth, and an appreciation of which will enable us to explain why the starvation causes acidosis bodies to appear in some cases and not in others.

For the thorough combustion of fat in the animal body a certain amount of carbohydrate must be simultaneously burned. Fat evidently is a less readily oxidized foodstuff than sugar; it needs the fire of the burning sugar to consume it. If the carbohydrate fires do not burn briskly enough, the fat is incompletely consumed; it smokes, as it were, and the smoke is represented in metabolism by the acidosis bodies. Such a closing down of the carbohydrate furnaces may be brought about either by curtailment of the intake of carbohydrates, as in starvation, or by some fault in the mechanism of the furnace itself, as in diabetes.

Fundamentally, therefore, acidosis is due to the same cause in starvation and diabetes, namely, to an improper adjustment between the metabolism of fat and carbohydrate. Besides fat, portein may also contribute to the production of acidosis bodies when carbohydrate combustion is depressed. Bearing these principles in mind, it is easy to see how the intensity of acidosis which develops during starvation will depend upon the relative metabolism of carbohydrate on the one hand and of fat and protein on the other; it will therefore depend on the amounts of these foodstuffs which have been stored in the organism, and this again will depend on the nature of the diet previous to the starvation period. For the first few days following entire abstinence from food in a healthy, well-nourished individual, very little if any acidosis bodies will be excreted in the urine, because the carbohydrate stored in the body as glycogen has sufficed during this time to maintain the proper proportion between fat and carbohydrate. Afterwards, however, the appearance of acidosis bodies is to be expected, because the glycogen stores become exhausted long before those of fat. If starvation be still further prolonged, a stage will come when the fat, as well as the carbohydrate, is used up so that the organism has now to subsist on protein alone. When this stage arrives, the acidosis will diminish, for although from certain of the amino acids of which this is composed acidosis bodies might be derived, yet this does not actually occur, because a large part of the protein molecule (nearly half) also becomes changed into dextrose, which by burning, as above explained, prevents the formation of acidosis bodies from the other part of the molecule. For the same reasons, marked acidosis will not be expected to occur during any stage of starvation in lean persons, who from the start must utilize mainly their stored protein to supply the fuel upon which to live.

In diabetes exactly the same principles apply, but to an organism in which the ability to metabolize carbohydrate has been depressed, so that "the maximum rate at which dextrose can be oxidized is fixed at some level which is absolutely

lower than in health." Therefore, since a certain proportionality must exist between the rates of combustion of fat and carbohydrate, the diabetic can thoroughly oxidize less fat; in other words, an amount of fat which could readily be burned in a healthy body is improperly burned by the diabetic, and acidosis bodies accumulate.

"In order to check a diabetic acidosis, it is necessary to restore the proper ratio of fatty acid to dextrose oxidation," which can best be done by starvation, rest in bed and warmth. But this treatment may not at first suffice, because we have to deal not only with the acidosis bodies derived from fat but with those which can be derived from protein on account of the diabetic organism having lost the power even of burning the dextrose which is derived from this foodstuff. By persistence in the starvation, however, the ability of the organism to utilize carbohydrate usually becomes so far restored that enough burns to prevent acidosis. Every case of diabetes cannot therefore be expected to react in the same way to starvation, the determining condition being the relative quantities of glycogen and fat stored in the body at the outset of the fasting period, and this again on the nature of the previous diet.

To sum up, "fasting will lower acidosis either in health or in diabetes if it has the effect of stopping a one-sided metabolism and throwing the tissues on a more nearly balanced ration of fatty acids and glucose." A practical point may be noted here, namely, that there is likely to be more danger of serious acidosis developing during starvation in the case of fat than in lean diabetics.

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—J. J. R. M.

Chloroform Necrosis of the Liver

THE fact that chloroform may produce important changes in the organs of the body, particularly in the liver, is a significant one which is based upon considerable clinical and experimental observation. That ether and other narcotics are less apt to produce the same changes is also well known. The essential of chloroform poisoning is severe intoxication associated with anatomical changes which resemble those of acute yellow atrophy, phosphorous poisoning, eclampsia, certain septicemias, and acute syphilis.¹ These changes have been supposed to be the results of local defective oxidation in the affected organs. Wells believes that the chloroform damages the liver cells in such a manner that while their general activities are lost, the intracellular proteolytic enzymes are not interfered with and so produce autolyses. The intoxication results from incomplete hepatic function.

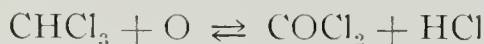
In connection with this matter of chloroform necrosis, Graham² brings apparently conclusive evidence that in chloroform poisoning "the liver necrosis

¹For general account of these conditions see Wells' *Chemical Pathology*, 1914, p. 49 et seq.

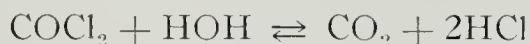
²Graham: *Jour. Exp. Med.*, 1915, xxii, p. 48.

is produced chiefly by the action of acid (largely probably by hydrochloric acid which is formed in the metabolic destruction of chloroform), and this ability to produce liver necrosis is a general property of the alkyl halides, all of which probably yield halogen acids in their breakdown in the body." Graham calls attention to the ease with which chloroform produces necrosis as compared with ether, and to the probability that that difference depends upon either different molecular effects or to different products which are formed in the metabolism of the two substances.

When chloroform is oxidized, the reaction results in the formation of phosgene and hydrochloric acid:



When phosgene in the presence of water reacts to form carbon dioxide and hydrochloric acid,



It is evident then that when chloroform is oxidized in the presence of water, 3 molecules of hydrochloric acid are produced, for each molecule of chloroform present.

With these facts as a starting point, Graham studied the tissue changes produced by hydrochloric acid, by chloroform and other substituted products of methane and by other alkyl halides. He has found that while certain narcotics produce these changes, others do not, and that all the substances, narcotic or otherwise, which do cause them are those which on splitting give rise to halogen acids. Certain substances such as chloral, which resemble in a general sort of way chloroform, produce no necrosis because they do not produce, on oxidation, the proper acids. Moreover, the activity of the substances in producing necrosis runs parallel with the amount of halogen acid produced during metabolism. For all of these statements there is abundant experimental support, even for the presence of acid in the areas of necrosis.

Graham wisely insists that the production of halogen acids is not the only factor in the production of necrosis, but that they are important ones. He calls attention to the fact that when disturbances of oxidation result, other acids (organic) are apt to accumulate—notably lactic acid, and this acid is able to produce profound tissue changes. It is possible that in phosphorous and arsenic poisoning, for instance, it is the organic acids, which accumulate because of decreased oxidation, that cause the anatomic changes. In chloroform poisoning, or that produced by the halogen narcotics, he seems to prove that the halogen acids are the essential factors.

Graham has not only made this contribution but has followed it up therapeutically and shows that the administration of alkalies is able to inhibit the production of the lesions. He used the alkali-salt solution of Fischer. He says in conclusion,—

1. After the administration of some of these drugs, there has been noted

an increase of neutral salts of the halogen acids in the urine, a fact which indicates that the corresponding halogen acids must have been formed somewhere in the body.

2. The necrosis-producing powers of dichlormethane, chloroform, and tetrachlormethane parallel the amounts of hydrochloric acid which these substances theoretically can yield in their breakdown outside the body. Likewise, the power to produce tissue changes exhibited by the ethyl compounds, varies directly with the ease with which they form their respective halogen acids *in vitro*.

3. Ether and chloral hydrate do not yield halogen acid in their breakdown in the body likewise do not produce necrosis. They induce only edema and fat infiltration to a less marked degree.

—P. G. W.

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ORIGINAL ARTICLES

IMMUNITY IN SYPHILIS*

BY HANS ZINSSER, M.D., NEW YORK CITY.

I.

UNTIL relatively recent years there seems to have been little question in the mind of clinicians regarding the existence of true immunity in syphilis. It was stated uncompromisingly by Ricord that "an individual who had once acquired syphilis was thereafter protected against reinfection." This opinion acquired wide acceptance and was shared by most of his contemporaries. Bäumlér in 1875 summarizing the authoritative opinions of that period stated that "One who has once had smallpox, scarlet fever, typhus, etc., is, as a rule, not liable to these diseases again for the rest of his life. The same is true of syphilis." However, even at the time Bäumlér wrote this, exceptions to the supposed rule were accumulating and he appends, to the positive statement given above, references to observed instances of second infection reported by Bidenkap, H. Lee, Diday, Köber, Zeissl, and others.

The conception of the existence of a true acquired immunity was also expressed and widely accepted in the so-called "laws" of Colles and Profeta. The former, first enunciated by Beaumès and later, in 1837, stated by Abraham Colles, of Dublin, is the well-known generalization based on the observation that mothers who have borne syphilitic infants were not infected by their children while suckling them, although such children might often infect wet nurses. Profeta's observation was the converse of this, namely, that children born of mothers who suffered from active syphilis during the period of conception did not acquire the disease from their mothers.

Both of these phenomena appear too well founded in clinical observation to be questioned as, at least frequent occurrences. Moreover, considering the intimate contact between mother and infant during the first months after birth, they acquire unusual importance. However, as we shall see, they have been deprived of much of their bearing as proofs of true acquired or inherited

*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

immunity by serological investigations such as those of Bauer, Knöpfelmacher, and others, which have shown that mothers of syphilitic children usually give positive Wassermann reactions, a fact which makes it seem likely that such women are suffering from syphilis in a latent form and are not immune in the ordinary sense. This indeed was the interpretation given to the "laws" of Colles and Profeta by Fournier and by Matzenauer at a time prior to that at which serological data were available. It is still unclear why such mothers should so frequently exhibit the disease in a latent form. However, this is a matter for the intelligent discussion of which we are not at the present time in the possession of sufficient information.

In the years just preceding the period of experimental investigation of syphilis upon animals much purely clinical research was carried out on the problem of reinoculation and what is commonly known as "superinfection" of syphilitic human beings. Much of this work is unavailable as scientific evidence owing to the difficulties of distinguishing, at that time, between the true chancre and the chancroid, but a considerable number of the observations then made have been of much value in pointing out directions of later research upon animals. The work has been so thoroughly analyzed both by Neisser and by Levaditi that it would be needless repetition to do so again. The records include both accidentally occurring superinfections, and purposeful experimental reinoculations. Without, therefore, going into details concerning the individual cases we may summarize the conclusions justified from a study of these observations.

1. The reports of Lasch, Jadassohn, Sabearéanu, Queyrat, Taylor, H. Lee, Knowles, and many others, have shown that patients are susceptible to a second inoculation during the first incubation time, that is, during the period elapsing between the first infection with syphilis and the appearance of the chancre. Second positive inoculations have also been successful at periods shortly subsequent to the appearance of the primary sore.

Autoinoculations and reinoculations undertaken after the chancre has become well developed have been, in the main, negative, though Queyrat reports a case successfully inoculated daily up to the eleventh, and Taylor one inoculated on the fourteenth day after the appearance of the primary induration. In contrast to these and other successful attempts, many observers record failure. However, the point is not one of particular importance, since, after all, the appearance of the chancre does not mark off any fundamental change in the progressive pathological development of the disease, and indicates only the completed reaction at the point of entrance. The fact remains that analysis has revealed that reinoculation, with the appearance of the second initial lesion, is possible up to about the twentieth to the thirtieth day after the first infection with the treponemata (Mauriac and Neisser state the twenty-second day, Queyrat cites a case eleven days, Linderman one twenty-four days after the appearance of the chancre). It is claimed by some of the observers, however, that even when reinoculation during this period is successful, the incubation of the second and subsequent lesions is shorter, that the induration itself is less severe, may not ulcerate, and heals more readily than the first.

In judging of the success or failure of reinoculation practiced during the

later days of the period above referred to, the possibility must be borne in mind that the trauma produced at the inoculation might have served to favor the development of a localized focus, the treponemata at this time being very probably well distributed throughout the body. Unsuccessful control inoculations with non-syphilitic materials in Queyrat's cases would tend to eliminate this possibility, whereas lesions resulting at the sites of such control abrasions in the experiments of Neuman and Cehak, and of Levaditi, appear to support it. However, this is not of great importance inasmuch as it determines merely a relative lengthening or shortening of the period during which reinoculation or superinfection is possible.

2. After the disease is well established as a systemic infection, that is, from the time of development of the chancre throughout the so-called active "secondary" period, reinoculation is either impossible or, at any rate, extremely difficult. Neisser cites Rollet as follows:

"Although I and my predecessors have a thousand times attempted to reinoculate luetic subjects, we have never observed a successful case. I know no single fact more thoroughly proven than the insusceptibility of a syphilitic to the action of a new virus, and, moreover, these experiments are so harmless that they may be performed without scruple."

The same opinion was held by Mauriac and is in a general way assented to by Neisser in his summary of this place of his studies. (Neisser, *loc. cit.* pp. 180-181.)

That the patient with well-developed lues has acquired a considerable degree of resistance to fresh inoculations is pretty generally accepted, therefore, by all who have studied the question—but there are investigators, notably Finger and Landsteiner, who believe this resistance to be less absolute than stated by earlier writers. Their observations on patients with active syphilis seem to indicate that superinfection is possible "under certain circumstances in all stages of the disease," but "the positive effect can be obtained only with considerable quantities of the virus." Furthermore, Landsteiner states that, in general, lesions so obtained are relatively slight in severity, do not appear as primary indurations, but have the tendency to simulate the particular variety of lesion spontaneously manifest in the individual at the time. Were it not for Finger and Landsteiner's monkey experiments, to which we will refer directly, and which seem to bear them out in their interpretation of these inoculations as "positive" results, the simulation of the spontaneously occurring lesions by the inoculation-products would again justify suspicion that these experimental results represented merely traumata in which, as points of less resistance, the patient's pre-existent disease had found a favorable spot for localization.

Observations in this respect, also, are corroborated by the older literature. A report which has direct bearing on this point, is one of Queyrat and Pinard who inoculated a tertiary patient with chancre material, obtaining not a primary sore but an ulcerated lesion having the clinical characteristics typical of the late skin manifestations of the disease. The autoinoculation experiments of Ehrmann also would tend to show that the resistance of the luetic subject is a relative one only. Ehrmann succeeded in producing positive autoinoculation-products in 45 syphilitics with papular eruptions. Control inoculations with

sterile water were negative. Although his observations and those of Finger and Landsteiner, with other similar ones, teach us that superinfection during the disease is possible the nature of the lesions, their short incubation time, and their exceptional character when averaged with the total of such attempts, prevent them from invalidating the conclusion that there is a resistance at this stage higher than that of the normal subject.

We may conclude, therefore, concerning the secondary period of the disease, that the luetic individual has acquired a resistance which while not absolute is at least very high, and protects him from fresh external inoculation, although at the time his disease may still be progressing and in no sense overcome.

3. During the late stage of syphilis, the stage at which, according to the more or less arbitrary divisions of Ricord, we are accustomed to speak of it as "tertiary," the resistance is still manifest, though apparently not so regularly potent as during the preceding "secondary" period. Neisser expresses himself with great caution and accepts few, if any, of the observed reinoculations of tertiary cases as surely representing unquestionable freshly acquired infections. Nevertheless, taking into consideration a detailed study of individual reports, he concludes that resistance during the late stages is pronounced but already beginning to wane. He himself in the *Festschrift* to F. Joseph Pick in 1898 cites a case of the development of a chancre in a tertiary case which, however, was not followed by constitutional symptoms.

4. In the preceding paragraphs we have concerned ourselves entirely with questions of "superinfection," that is, the implantation of the syphilitic virus into subjects still suffering from manifestations of the disease. A problem of equal theoretical, and of much greater practical importance, is that dealing with true "reinfection." By "immunity" in the ordinary sense, we mean an increased resistance to specific infection which persists for a more or less prolonged period after the active disease has been overcome and the causative agents removed from the body. It is not easy to draw conclusions concerning this point from observations on human beings, since it is most difficult to decide, even with the aid of serological methods, whether or not a given case is cured in the bacteriological sense; for syphilis is pre-eminently the disease in which there occur frequent and prolonged latent periods terminated, often after lapses of years, by the reappearance of foci of a grave nature.

Moreover, our own experiments both with syphilis in rabbits and the treponemata in culture have convinced us that these microorganisms may assume for long periods a condition of metabolic latency, a sort of resting period, during which they incite no reactions of any kind on the part of the tissues, apparently do not multiply to any great extent and yet remain alive and capable of development when conditions favor this.

In spite of these difficulties, however, careful clinical studies by Jonathan Hutchinson, Taylor, Hudélo, Neisser, and many others, have furnished data which warrant an opinion upon this problem. Of especial value is the painstaking analysis of reported cases of reinfection in syphilis made in 1909 by Felix John.

In contrast with some others who have attempted similar analyses John

takes the utmost care to separate these cases into the ones in which the evidence of true reinfection is absolute, and those in which the reported details are insufficient to exclude the possibility of recrudescence. In agreement with Taylor he insists upon a symptom-free interval of five years between the last manifestations of the first attack and the appearance of the second. As an example of what he calls an "Ideal Fall" we may cite the following:

X. J., April 1, 1872, primary sore.

Roseola, polyadenitis, mucous patches. September, 1872, papular rash. March, 1873, palmar and plantar spots, iritis. 1875, gumma of tibia and sepriginous syphilide of right thigh.

Four courses of inunctions and KI.

1876, married—two healthy children.

No symptoms till 1887 when he acquired a second chancre followed by typical roseola. In 1888 wife had still-birth.

John has analyzed in this way 356 cases of supposed reinfection, in 34 of which the first attack was of congenital origin. Of the remaining 322, fourteen were cases which seemed unquestionable instances of reinfection and in 16 more there was practically no doubt of this. Of the 34 hereditary cases there were three, one of Emery, one of Taylor, and one of Hochsinger, in which there was practically no question of their nature as valid reinfections. In all of the others there was one point or another which rendered them doubtful as evidence.

John concludes that true reinfection can unquestionably occur in syphilis but that it is relatively rare.

To John's cases Neisser has added others reported between 1909 and 1911. Yet even with these, the total number is not a large one. Nevertheless, we should not be tempted to conclude from this that the relative infrequency of such cases is evidence in favor of the existence of a true immunity analogous to that following typhoid, plague, etc. For we have seen that a very definite insusceptibility is coincident with the persistence of actual disease in the subject and, as Neisser points out, the more recent investigations carried out with the aid of serological tests have shown that the number of cases uncured though long without symptoms, is much larger than formerly supposed. The scarcity of true reinfection, therefore, may well be due to the relative scarcity of completely cured cases. Moreover, it must be remembered that, in this disease, even when final recovery results, it is usually achieved only at an age when the individual is less exposed to reinfection because of changed economic and domestic conditions, or by reason of the virtue which comes with arteriosclerosis and the wisdom of the burnt child that fears the fire.

Granted, then, that true reinfection is possible, is there any evidence that when it does occur, the second attack is less severe and more easily cured than the first, a fact which would also tend to support the opinion that a certain degree of immunity persists. Jonathan Hutchinson expressed this view in a clinical lecture in which he states that "second chancres are far more common than second attacks of constitutional syphilis." However, John in his summary, in which Hutchinson's cases are included, finds that in general the disease has run a second course very similar as to severity to that incident to the first infection.

If we gather together, then, the facts revealed by clinical study we may conclude with Levaditi, Neisser, and most others, that:

1. The syphilitic subject acquires definite resistance to reinoculation which becomes manifest soon after the appearance of the primary sore, at a time when the virus may be regarded as having gained universal systemic distribution.

2. This resistance, high though not absolute, persists throughout the secondary or most active period of the disease and into the tertiary stage. During the latter, however, it appears somewhat to decline, reinoculation or superinfection being more frequently possible at this period.

3. When syphilis is entirely cured, susceptibility may in all probability be regarded as returning, possibly, though not certainly, to the same degree as it exists in the normal subject. The reasons for this last belief will become more clear when we study the evidence contributed by animal experimentation.

II.

Notwithstanding the admirable thoroughness with which clinical data had been collected and analyzed in the study of syphilis, progress beyond the points indicated in the preceding sections was quite impossible without the aid of animal experimentation and a knowledge of the causative agent. Fortunately these two deficiencies in our methods were removed when in 1903 Metchnikoff and Roux succeeded in transmitting the disease to a chimpanzee, and in March, 1905, Schaudinn discovered the *treponema pallidum*.

As a matter of fact, probable transmission of syphilis to lower monkeys had been accomplished as early as 1879 by Klebs and subsequently by Neumann, Martineau, and Charles Nicolle, but in none of these experiments had it been possible to prove beyond question the syphilitic nature of the inoculation-products. In the chimpanzees inoculated by Metchnikoff and Roux, the animals developed not only primary sores but also secondary eruptions, polyadenitis, and enlarged spleens in such characteristic manner that the identity of the inoculation disease with human syphilis could no longer be doubted.

Successful transmission to other anthropoids and to lower monkeys were then announced, in rapid succession, by Metchnikoff and Roux, Ch. Nicolle, Neisser, Baermann and Halberstaedter, Finger and Landsteiner, Hoffman, and others. The susceptibility of monkeys was tabulated by Neisser in the following series: Chimpanzee, Gibbon, Orang-Outang, *Cynocephalus babuin*, *Cynocephalus sphinx*, *Cynocephalus hamadryas*, *Cercopithecus fulginosus*, *Macacus niger*, *M. nemestrinus*, *M. cynomolgus*, *M. sinicus*, *M. speciosus*, *M. rhesus*. In 1906 Bertarelli produced syphilitic keratitis in rabbits, demonstrating the *treponema pallidum* in sections of the cornea and in 1907 Parodi first produced syphilitic orchitis in the same animals.

Apart from monkeys and rabbits, no animal species have so far been shown sufficiently susceptible to be available for systematic study. It is true that the production of keratitis is claimed in dogs and sheep (Bertarelli, Hoffman and Brünig), in guinea pigs (Bertarelli), in cats (Levaditi and Yamanouchi), and in goats (Bertarelli). However, these experiments have been isolated and too uncertain with present methods to offer material for experimentation. Our own attempts on cats, pigs, guinea pigs, rats, mice, and a few birds, have yielded

negative results only. There are many observations of great scientific interest which might be discussed in connection with the problem of susceptibility of various animal species, however, we will confine ourselves at present to those phases of the work only which have bearing on the questions of immunity.

In the fundamental premises, the work on monkeys has pretty accurately confirmed the observations concerning reinoculation and superinfection previously made on human beings.

The most extensive studies in this respect are those of Neisser and his associates in the Javan expedition. Briefly stated, Neisser reinoculated 135 animals 165 times with negative result on the second and subsequent inoculations. The second inoculations were made at periods ranging from 21 days to two years after the first. In but 27 animals did the reinoculation show positive results, and in ten of these cases only does Neisser recognize the experiments as valid. Although these ten positive reinoculations it is true, add an element of irregularity to the series, they constitute but 6.8 per cent of the entire number, a proportion which in no way invalidates the experiments when we consider that the work was done entirely on the lower monkeys, animals that are far less susceptible to syphilis than are human beings and in many of which, therefore, systemic distribution of the virus (a generalization apparently necessary for the development of resistance) may not have taken place. Neisser's conclusions, therefore, that monkeys, like human beings, are not reinoculable while suffering from systemic syphilis, seem entirely justified.

His work, as well as that of Finger and Landsteiner, and of Kraus and Volk on lower monkeys, has shown that resistance does not develop until the twelfth to the twentieth or twenty-first day after the first inoculation; that is, again as in man, when the virus has become generally distributed. Finger and Landsteiner, furthermore, noticed that reinoculation-products, obtained by reinfection during the first incubation period; that is, before the development of the primary lesion, were less severe and developed in a shorter time than did the first lesion. This phenomenon which would tend to mark another analogy to the conditions prevailing in human beings, was not observed in the experiments of Neisser and of Kraus and Volk. However, like the similar observation in human beings, it seems to indicate the gradual acquisition of resistance as the virus begins to exert its influence upon the tissues.

Again, with monkeys as in man, the question arises whether the resistance so unquestionably proven is a condition merely coexistent with active disease, or whether it may be interpreted as a true immunity which persists after the microorganisms have been completely removed. The most directly pertinent experiments are those of Neisser. Neisser reinoculated monkeys at periods ranging from 27 to 645 days after the first infection. After waiting a time sufficiently long to insure the negative result of the reinoculation, he used organ-substance from these animals to inoculate other monkeys. In 22 experiments of this kind he obtained positive results—showing that the organs of the apparently immune animals still harbored virulent *treponemata*. In seven animals only did the inoculations with organ-substance fail to produce lesions, but of these all but one died before the 30th day after inoculation.

In contrast to these results Neisser found that animals which had been

"cured" by various treponemacidal agents such as Atoxyl, Arsacetin, etc., were almost regularly reinoculable. In fact, these experiments were so uniform that Neisser later utilized the reinoculation method as an index of cure or persistence of the disease.

The results obtained in monkeys, therefore, are very similar to those determined by clinical observations in man, and the following statements may be taken as summarizing the conditions revealed by monkey experiment.

1. That the body develops resistance, progressively increasing as the virus becomes generally distributed.
2. That the resistance probably reaches its highest development during the early tertiary period.
3. That complete cure is probably synonymous with gradual return of susceptibility.

The only other animals on which systematic experimentation has been possible up to the present time, have been rabbits. Since Bertarelli's successful production of keratitis and Parodi's inoculation of the testes in these animals, they have been studied carefully by a number of workers, chiefly Uhlenhuth and Mulzer, and Noguchi; and in our own laboratory, with Hopkins and McBurney, the writer has observed rabbit syphilis for a number of years. From a large mass of observations it appears that the conditions in rabbits are not identical with those observed in man and monkeys. So far, the testes and the eyes are the only organs in these animals in which syphilitic lesions can regularly be produced, and although *treponema pallidum* may apparently be distributed generally to the organs after inoculation, it does not easily arouse pathological reactions except in the organs named, and the lesions produced are not accompanied by a generalized resistance comparable to that discussed in connection with the higher animals in preceding sections of this paper.

Bertarelli found that he could reinoculate the cornea in a rabbit that had previously been inoculated with syphilis. Uhlenhuth and Mulzer, and Neisser and Pürckhauer, showed that infections of the eye could be produced while the opposite eye was still syphilitic. Tomazewski found that scrotal infection did not protect against infection of the cornea, and vice versa. Furthermore, Uhlenhuth and Mulzer, on the basis of a very thorough study, believe that such reinfections are neither less extensive nor more rapid in healing than was the first lesion. The same authors noticed resistance to reinoculation in two animals only, and these were young rabbits in the first weeks of life, which, they believed, had been generally syphilized by intracardial injections. Interesting in this connection are claims by Ossola and Truffi who believe that successful skin inoculation in rabbits confers a certain amount of skin resistance and this is in harmony with the belief of Kraus and Volk, that a specific skin immunity in syphilis is possible. Of great interest to us and of a possible theoretical bearing which we will discuss below, are observations made by the writer with Hopkins and McBurney on 20 rabbits which were reinoculated into the testes after apparent healing of lesions in these organs. It appeared that in rabbits the opposite testis can be successfully inoculated before, during, or after, the existence of a testicular lesion on one side, but that reinoculation of the same

testis which had apparently returned to normal, at periods ranging from 6 weeks to one year was not often successful. There is a certain amount of evidence here of a purely local immunity, a matter which we will discuss at greater length presently. Perhaps the difference between rabbits and the higher animals lies chiefly in the fact that syphilis is not generalized in the same sense that it is in man and monkeys, and even though inoculations from the organs of syphilitic rabbits may often result positively, this might signify only that the treponemata have been generally distributed by the blood stream and latently lodged in the organs, without, however, arousing in the tissues any sort of pathological response. This idea would seem to be borne out by the two experiments of Uhlenhuth and Mulzer cited above in very young rabbits since in such animals true generalization seems to be more common. The study of very young rabbits will be continued with this point in view.

It is apparent from the preceding considerations that resistance to syphilis differs from that acquired in many bacterial infections chiefly in the fact that it does not persist after the disease is over, but probably coexists only with the presence of the living incitants in the body. In this respect it seems to be similar to the conditions prevailing in many protozoan diseases. Thus Schilling cites a case of *Trypanosoma Brucei* in a steer, experimentally infected by Koch, in which reinoculation was repeatedly negative, this result being at first falsely interpreted as immunity; but six years later Kleine found that the same steer still harbored the trypanosomes in his blood—showing that the resistance to reinoculation was not, in this case, an evidence of true immunity, but rather represented a condition of insusceptibility to “superinfection” analogous in every respect to that existing in syphilis. Similar conditions have been shown to prevail in Texas fever and Schilling believes that they may be regarded as also existing to a certain extent in Malaria and Sleeping Sickness. In both of these conditions complete spontaneous sterilization of the body (without medicinal aid) is probably rare, possibly does not occur at all, and the apparent immunity to reinfection is, as in syphilis, an evidence of persistence of the disease in a latent form.

In weighing the analogy of syphilis to such protozoan diseases, one is inclined to wonder whether syphilis in man may be regarded as at all spontaneously curable. So few are the cases left untreated and so rarely does the reinfection occur even in the face of specific remedies, that it seems to us more than likely that in syphilis, as in Sleeping Sickness, a spontaneous “sterilizing” immunity does not occur. This is a point, however, regarding which it is impossible to gather data.

In order to distinguish the conditions outlined above tersely from the ordinary conception of immunity, Neisser speaks of the alterations which govern the reactions of the luetic body to freshly introduced virus as “Anergie;” and “Umstimmung” or “*Allegerie*.” By “*Anergie*” (a term first used by v. Pirquet and subsequently introduced by Siebert in working out the analogy between pigeon-epithelioma and syphilis), Neisser designates a condition of inability to react by cellular change to contact with the virus. As he uses it, it implies a passivity on the part of the invaded tissues (in which “*die Zellen auf die Spirochaeten schwer oder garnicht reagiren*”), by which there is not necessarily a destruction

of the invading treponemata, and which, therefore, cannot in any sense be interpreted as a "Schutz wirkung." By the "Umstimmung" of Neisser, the "change-ment dans la mode de reaction" of Levaditi, is meant the changed reaction capacity of the syphilitized tissues which determines the characters of the lesions at various stages of the disease. Thus it is obvious that cellular reactions which result in the primary induration are quite different from those which produce the tertiary gumma, and that the histological changes of the roseola are distinct from those of the serpiginous syphlide of the late stages. And since, as we shall see, there is no valid reason to assume that the incitant has been modified in virulence or vitality, we are forced to believe that the reaction capacity of the body cells has been altered.

III.

Since it is a fact, then, that syphilitic infection so changes the body tissues of man and monkeys that, during its course, resistance to reinfection is produced, it should be possible to analyze this resistance into its responsible factors and perhaps utilize the knowledge so gained for practical therapeutic purposes. Before we proceed to do this, it will be of advantage to review briefly the attempts at active and passive immunization which have been made in animals and man.

Metchnikoff and Roux followed their first animal inoculation studies by extensive vaccination experiments. Some of their first work along these lines is perhaps marred to some extent by an insufficient recognition of the resistance which depends upon persistence of the disease rather than upon true immunity, but a good many of their observations are of fundamental importance. It will be convenient to classify their work and that of others into experiments dealing with "active" and those dealing with "passive" immunization.

Metchnikoff and Roux first worked with filtered virus and virus killed at 51° C. All their attempts with such material were negative in that the monkeys treated with it could not be regarded in any sense as immunized. In their reports of these experiments they remark that they believed this to be due to an absolute loss of power to incite reaction on the part of the vaccine-material. We emphasize this point here because our own subsequent work inclines us to believe, with them, that the production of a reaction is necessary for the development of any considerable degree of resistance.

Neisser carried out a large number of attempts at vaccination in which he used extracts of syphilitic primary lesions and of the organs of congenitally syphilitic children, killed by the addition of carbolic acid. Unfortunately he assumed that his extract contained syphilitic antigen because it gave positive reactions by the complement fixation technique of Wassermann, an assumption which we of course know now to be unfounded as far as any relation to the body substance of the treponemata is concerned. This, to our mind, deprives these particular experiments entirely of their negative importance.

In rabbits a large amount of work has been done by Uhlenhuth and Mulzer. They injected living material from rabbit lesions intravenously and subcutaneously, without ever observing any evidence of protection against subsequent inoculations.

Of perhaps the greatest importance in connection with active immunization are the attempts made upon human beings by different investigators.

Casagrandi and de Luca tried prophylactic immunization on six human beings by injection of filtrates obtained from primary lesions. Two of these people later contracted syphilis in the ordinary way.

Possibly the most hopeful results are those obtained by Spitzer by a method suggested by Kraus. Kraus, reasoning from the fact that syphilis like hydrophobia was a disease with long incubation time, expressed the hope that perhaps the method of Pasteur in hydrophobia, that is, active immunization during the period of incubation might, in syphilis also, tend to abort the disease. Accordingly, Spitzer treated 15 cases of early syphilis immediately after the appearance of the chancre by subcutaneous injections of emulsions made from human chancre material in dilutions of 1:200 to 1:20. The cases received from 11 to 20 injections and in seven of them the disease was uninfluenced. In the others, however, subsequent symptoms were delayed, and in four, no generalized symptoms occurred. In a later communication, Spitzer reported 23 further cases similarly treated, 10 of which failed to develop generalized symptoms and in 9 of these the Wassermann test remained negative. One of them, a fact which is of great importance, was spontaneously reinfected with syphilis two and one-half years later. These results if accurate in every way, are of the greatest importance, but are diametrically opposed to the experience of all other investigators. Monkey experiments along the same lines by Neisser gave entirely negative results and Brandweiner as well as Kreibich were unable to confirm Spitzer's results in man. Further comment on the Kraus and Spitzer method is valueless without more experimental data. It is one of the few rays of hope but so isolated that one is forced to skepticism. Metchnikoff and Roux did almost the identical thing in an orang-outang and obtained lesions both at the point of the original inoculation as well as that at which the subsequent "protective" injection was made.

The only experiments in which an attempt at vaccination with *attenuated virus* was made with some indication of efficacy, is one of Metchnikoff and Roux, the outcome of an accidental laboratory infection. It appears that a laboratory assistant who had been attending to the animals, noticed a small lesion on his lip which did not look like a typical syphilitic chancre. In order to allay the patient's fears, however, Metchnikoff and Roux did inoculations from the patient to monkeys and these were positive. Nevertheless, Fournier after examining the original lesion declared it so unlike the ordinary primary sore that he did not advise treatment. No secondaries developed in the patient nor in the three chimpanzees inoculated with the material. From this occurrence Metchnikoff and Roux concluded that the patient had probably been infected in handling the monkeys, and that the virus had become attenuated by passage through these animals. On the basis of this observation they later inoculated a willing subject 79 years old with virus carried for five generations in lower monkeys. The lesion which developed was very slight, consisting only of a local induration and no generalized symptoms developed. A previous attack of syphilis Metchnikoff believes could be reliably excluded in the subject. The experimenters suggest that the passage through lower monkeys may attenuate the virus for man, this leading to relative immunity to subsequent inoculations, and furnish a possible means of protection. Their experiments are too few to

permit conclusions as yet, but even should they hold good, the method would seem to imply a considerable degree of risk and our experience with superinfections and reinfections in syphilis does not encourage the hope that a method so drastic would be justified when the benefit to the patient is apt to be of such duration only.

The history of passive immunization is an extensive one and hardly worth going into with any degree of detail since many times extravagant claims have been made only to be refuted by accurate study. There is a great similarity in respect to this between syphilis studies and those in tuberculosis and cancer. A brief examination of the bibliography in Neisser's book is sufficient to convince one of the many attempts that have been made in this direction and often by methods as ludicrous as the claims of success for which they formed the basis. The most careful and skillful workers have uniformly reported failure. Metchnikoff and Roux treated various monkeys with blood from syphilitic patients and used the serum of these animals for protective experiments. There are a few instances in which mixtures of such serum with syphilitic virus rendered this inactive on inoculation. A powder made of this serum was supposed to have some protective effect when applied to fresh inoculation spots within the first hour after inoculation. However, injection of the serum had no effect whatever. Casagrandi and de Luca using serum of a dog treated with syphilitic virus, obtained entirely negative results, and Finger and Landsteiner report negative results with monkey blood in man. The most extensive experiments were again those carried out by Neisser and his associates. They were done by the treatment of animals with dead and living syphilis virus, organ extracts, with the blood of syphilitic man and monkey, and horses, sheep, and monkeys were used for the production of "immune" serum. In no case was there the slightest protective effect on the part of the serum either *in vitro* or *in vivo*, and the results of the experiment were unqualifiedly negative.

IV.

When the method of complement fixation was successfully applied to the diagnosis of syphilis first by Detre, and then by Wassermann, Neisser and Bruck, it was generally assumed that this reaction incidentally demonstrated that specific antibodies were formed in syphilis. As matters have developed, however, this point of view can no longer be maintained. It was soon discovered that the antigen used for these reactions by Wassermann and his associates derived its "fixing" constituent not from the body substances of the treponemata contained in the syphilitic organs, but from certain tissue extractives chiefly of lipoidal nature which could be obtained readily from normal as well as syphilitic tissues. Although Bruck and others who have occupied themselves with the theoretical basis of the Wassermann reaction, still maintain that a specific antibody may be incidentally involved, they admit that this is the less important factor in the reaction which depends chiefly upon the existence of lipotropic substances which appear in the course of the disease as metabolic products either of the body or possibly of the treponemata. This is a subject which we will deal with in extenso in another paper. It is sufficient for our present purposes to point out that, although to a slight extent specific antibodies may play a part

in the Wassermann reaction, this is certainly not the chief element or even a very important factor involved. The truth of this conception has been further confirmed by the work of Noguchi, of Craig and Nichols, of Kolmer, and ourselves, in which it has been found that antigens made with pure cultures of treponemata produced a complement fixation with syphilitic sera to a very limited extent only, and in our work in which we have been able to duplicate the Wassermann reaction in a large series of antigens made from the treponema cultures, we have found that similar results could be obtained with cultures of colon and typhoid bacilli identically prepared, the fixing power to a large degree depending upon the lipoidal constituents of the bacteria. Whatever the ultimate explanation of the Wassermann reaction may turn out to be (and this is a subject which it would not be profitable to discuss at length at this place), it cannot be maintained that as it exists at present, it can be interpreted as demonstrating the existence of true circulating antibodies, analogous to those found in bacterial diseases in the blood of syphilitic patients.

Early in the history of such investigations, Fornet with his collaborators Schereschewsky, Eisenzimmer, and Rosenfeld, found that when the sera of syphilitics were mixed with clear extracts of syphilitic livers similar to those used in the Wassermann reactions, precipitates formed which were not seen in similar experiments done with normal sera. This Fornet in a number of communications interpreted as showing the formation of precipitins in the course of syphilis. At almost the same time, L. Michaelis made similar observations giving them the same interpretations. The experiments of Fornet have not found universal confirmation, but even if such precipitin reactions can be occasionally observed, they do not indicate true precipitins for the same reasons that the Wassermann reaction does not demonstrate true complement fixation of antibodies. The antigens did not represent strong treponema antigens and Jacobsthal and others have shown that with the ordinary Wassermann antigens syphilitic sera can produce precipitations visible under the dark field, the basis of the complement fixation being, therefore, one of probable colloidal precipitation. The formation of true precipitins, therefore, has not been shown for syphilis.

Investigations of the effect produced upon virulent treponemata by the sera of syphilitic individuals have likewise been unsatisfactory. Hoffman and Prowazek reported in 1906 that the serum of syphilitics in the later stages produced immobilization of the virulent treponema pallidum, an observation which was confirmed by Zabolotny. Landsteiner and Mucha were not able to observe such immobilization, nor did they see any evidence of agglutination in such experiments. In a limited observation of our own, we have also failed to see any regular or distinct influences of this kind. It is not impossible that a slight difference may exist in this respect between syphilitic and normal sera, but even if it occurs, the action is feeble, irregular, and entirely insufficient to be interpreted as having much practical importance in the acquisition of syphilis immunity.

Treponemacidal experiments have been done by some workers also with negative results. As we have stated in another part of this paper, the attempt to treat syphilitic animals and man with the serum of syphilitics has led to no

reliable results, and no evidence whatever that can be accepted has been adduced to show that virulent treponemata may be killed by active luetic serum.

As far as any opsonic action is concerned, Levaditi in histological studies in congenitally syphilitic children has seen phagocytosis or at least intracellular localization of treponemata in the alveolar cells of the lung and in the parenchyma cells of the liver and kidneys. For reasons not entirely clear to us, he interprets the former as true phagocytosis and the latter as a penetration of the treponemata into the liver cells to the detriment of the latter. We ourselves have occasionally seen phagocytosis in sections of syphilitic rabbit testes, but in all cases the process was not a very active one and not much can be said about its importance at present. As a matter of fact, Hopkins and the writer, in studying the mechanism of the natural resistance of mice against syphilis, injected virulent organisms into the peritoneal cavities and observed the treponemata in peritoneal puncture fluid, alive, actively motile, and unphagocyted, though surrounded by masses of leucocytes, as long as three days after their injection. It seemed almost as though the natural immunity of such animals might be similar to the "atreptic" cancer resistance spoken of by Ehrlich. The treponemata did not multiply in the mice but though, naturally, diminishing in number, were apparently neither killed nor even inhibited in motility by the peritoneal exudates and, for several days, swam in and out among the accumulating leucocytes, often adhering to them peripherally but not taken up by them. Lack of entirely satisfactory methods of staining cells containing treponemata make it difficult to speak with certainty of the actual occurrences. But we gained the distinct impression that the treponemata were not actively injured or destroyed until they had spontaneously died out owing to lack of suitable environment, i.e., nutrition.

In the case of natural immunity at any rate, we do not think that phagocytosis by the mobile leucocytes plays a primarily important role. However, these experiments will need further elaboration.

The search for antibodies gained new vigor when the efforts of Schereschewsky, Mühlens, Hoffman, and especially Noguchi, had resulted in successful cultivation of treponemata from syphilitic lesions in man and animals. It was hoped that with the causative organisms isolated, immunization and a clear understanding of the antibodies in syphilis might yield practical results. Kolmer observed that cultivated treponemata were agglutinated in the sera of rabbits treated with culture material, and such agglutinins were produced in high potency by the writer with Hopkins. Subsequently in our own laboratory with Hopkins and McBurney, extensive experimentation on the production of antibodies with culture pallida were carried out.

We were able to show that not only were agglutinins formed by the immunization of rabbits with such cultures, but also that treponemacidal antibodies which were analogous to the ordinary bactericidal substances in such sera were present. Also, it was shown by cross agglutination and absorption experiments, that treponemata cultivated from various sources were related in group reactions.

Indeed, experiments done with cultivated treponemata (much facilitated by the discovery of a simple method of obtaining mass cultures of old strains)

seemed at first very encouraging in that animals immunized with the cultures responded by powerful antibody formation. It was a perfectly justified hope, therefore, that antibodies produced with these "attenuated" or rather "avirulent" strains might have some action on the virulent treponemata in luetic lesions. However, subsequent work in this direction disappointed such expectations. We may briefly review this work as follows:

The serum of rabbits immunized with "culture" pallida although potent against "culture" pallida, had no effect either in agglutinating the virulent organism from rabbit lesions, nor did it exert any protective influence when the virulent organisms were subjected to its action before injection.

Conversely, the serum of syphilitic rabbits showed but a very slightly increased agglutinating power for the "culture" pallida. This increase of potency in a few experiments was definite but very slight, a few of the syphilitic animals agglutinating as highly as 1:25 and 1:50, whereas most of the normal rabbits agglutinated in 1:10 and some of them 1:25.

Although Kissmeyer has recently reported that diagnostic use might be made of the fact that sera of syphilitic individuals agglutinated the "culture" pallida, we have tried this with a considerable number of cases and found that although the sera of tertiary syphilitics will sometimes agglutinate a little more highly than will the sera of normal individuals, yet many patients suffering from non-syphilitic diseases agglutinated as highly and almost as regularly as did the syphilitics.

We have come to the conclusion, therefore, as far as our work has gone, that in the syphilitic human being there is as little agglutinin formation against the "culture" treponema as there seems to be against the virulent organisms. If the slightly greater agglutinating power found in some of the tertiary syphilitics can be considered at all, the reaction is so feeble that it is negligible from the points of view either of diagnostic value or protective importance.

Furthermore, vaccination either intravenously or locally into the testis with cultures has thus far failed to protect rabbits against subsequent inoculation with virulent material, and passive immunization with sera produced with "culture" pallida has been without effect.

From all this it appears that the "culture" treponema has immunologically no relation to the virulent organism. It has lost its virulence completely, as six and more successive inoculations into rabbit testes have sufficiently demonstrated to us.

The sera produced by many injections of dead and living culture organisms, have no effect whatever on the virulent organisms in vitro, and vaccination with it does not protect against subsequent infection.

The luetin reaction is the only method by which the relationship between the two is demonstrable at all, and there too, we have to reckon with what is generally spoken of as the non-specific increased sensitiveness of the syphilitic skin.

Were it not for the production of lesions with cultures in their early test tube generations by Hoffman, and by Noguchi in a few experiments, one would be almost in doubt as to the identity of the virulent with the culture organisms..

V.

The reactions in syphilis between the invading microorganism and the invaded subject thus differ in certain fundamental premises from those prevailing in diseases caused by most bacteria. The *treponema pallidum* is an organism which, unlike many bacteria, is rarely subjected to the necessity of adapting itself to extra-corporeal existence during the interval between its passage from one host to another. It practically always infects directly, being inoculated from one human being to the next and has in consequence developed a very delicate parasitism not unlike that seen in certain trypanosome diseases of rats and that which we ourselves have observed in the well-known spirochaete infections of white mice. A considerable percentage of laboratory white mice have been found to harbor actively motile spirochaetes, often in considerable numbers in the blood and peritoneal fluid without there being any objective signs of illness in the animals. It is an instance of what has been spoken of by Bail as "infection without disease," and approaches what biologists speak of as symbiosis, except that the host in this case does not benefit in any way by the invasion. Even in the case of the mice, a certain amount of gradual injury, perhaps only metabolic, by the slow removal of nutritive material, is taking place. In syphilis the mutual adaptation may perhaps be less complete, a sufficient accumulation of the invaders and especially a mechanical injury of tissue cells, of closing of tissue spaces together with a certain amount of toxic action, leading eventually to pathological changes.

The virulent treponemata apparently do not arouse true antibody formation in any marked degree. When they have been cultivated and have become accustomed to the test tube conditions they entirely lose their virulence, are easily attacked by the active constituents of animal serum, and are probably amenable to phagocytosis. When such cultures, living or dead, are injected into animals they act like other specific protein antigens and incite the formation of antibodies. However, these antibodies have no effect whatever upon the virulent organisms.

In consequence, it cannot astonish us that all efforts at passive immunization with the sera of syphilitic man and animals, or with those of animals systematically treated with dead virulent materials, have been unqualified failures.

However, this does not preclude the theoretical possibility of active immunization or vaccination with such materials since, in this case, the antigen distributed from the points of injection might act upon tissue cells throughout the body. However, with the exception of the unconfirmed reports of Spitzer, all attempts to vaccinate either with dead virulent material, or with living and dead culture material, have been disappointing. The few experiments of Metchnikoff with virus "attenuated" by passage through monkeys have indeed seemed to indicate some possibility of approaching the subject from this direction, but these isolated observations have been very logically criticized by Neisser and should not bear too much weight. The observations were made on two cases only, both of them well along in life, and the validity of the important conclusions drawn rests entirely on the always problematical fulcrum of complete exclusion of previous syphilitic infection in the two subjects. Moreover, attempts in this direction would be fraught with a considerable amount of danger and it is there-

fore questionable whether experimentation along these lines is sufficiently promising to be justified. There is certainly no attenuation for man by passage through rabbits as has been sufficiently proven by a number of accidental infections, an instance of which in a laboratory Diener has been reported by Gracetz and Delbanco.

We may state, therefore, as safely summarizing our knowledge of the conditions in syphilis that the resistance which undoubtedly develops during the course of the disease is one which depends upon reaction to the living virus only, cannot so far be produced in animals by systemic treatment with dead treponemata, and does not express itself in the formation of significant amounts of circulating antibodies analogous to those observed in bacterial diseases. Moreover, it is a well-known fact that the treponemata can continue to do injury to many organs and tissues at a time when reinfection by the paths of skin and mucous membranes is no longer possible.

How, then, are we to explain this peculiar state of affairs? A clue to the problem we think is found in the 20 rabbits which Hopkins, McBurney and the writer inoculated into the testes after apparent recovery from a previous lesion. Ordinarily in rabbits, as we have stated before, no generalized resistance is developed during the disease, and the opposite testis can be successfully inoculated before, during, and after the existence of a lesion on the other side. In these rabbits it was found that testes that had apparently recovered from a previous lesion, were not subsequently as easily infected as were normal testes. It has seemed to us from this as well as from a careful study of the observations of other investigators, that resistance in syphilis was probably a matter of localized reaction. Tissues which have sustained active invasion with the living virus react and gain thereby a certain degree of resistance which expresses itself in a failure to react to subsequent inoculation. This would explain why in syphilis of the human being reinoculation is unsuccessful and reinfection of the skin and mucosæ does not occur spontaneously at a period later than the early secondary stages when the virus has become systemically distributed. It would furthermore explain why in this disease organ after organ may be pathologically involved when skin infection is no longer possible. This point of view is entirely in harmony, though perhaps from a slightly different aspect, with the skin immunity suggested by Kraus and Volk, where the resistance is attributed to the tissue as a whole rather than to a local cell group. The cells which have once reacted to the living virus no longer respond, i. e., can no longer be injured for an indefinite period after recovery. However, the factors which lend them this resistance, whatever they may be, are not distributed to the blood stream in a way analogous to that in which antibodies are mobilized in bacterial diseases, and the effect of the resistance of the local area is not distributed to remote parts of the body. It is of course likely that a certain amount of phagocytosis of treponemata by the now resistant fixed cells may account for the absence of local injury. This, however, we have not yet been able to prove sufficiently and further studies on this point are necessary. As far as any positive evidence can be adduced at the present time, the newly entering treponemata may not be entirely destroyed. It may well be that the tissues do not react and are in the state which Neisser calls "Anergie." The treponemata that enter during this period

may nevertheless remain uninjured and be as capable of subsequently causing lesions in other locations as are those already present in the patient. This phase is being studied by comparative histological observation on the fate of treponemata which have been injected into normal tissues and into tissues rendered resistant by previous infection.

In animals like monkeys and man where generalization is rapid and apparently complete, the resistance becomes a general one. In animals like the rabbit in which the lesion—or in other words pathological response, occurs in a few organs only, the resistance is limited to the particular organ or organs that have previously developed a lesion.

It must not be forgotten that such a resistance probably persists for a limited time only, and does not imply the sterilization of the body and the complete destruction of the microorganisms. These may, and probably do, remain alive and potent in various parts of the body, capable of again setting up new lesions in parts hitherto uninvolved or again susceptible after a diminution of their local, acquired resistance. That the virulent treponemata may remain thus latent, alive and virulent has been sufficiently shown in animal experimentation by successful inoculation with tertiary lesions and by the frequent late accidents, especially of the nervous system, in individuals apparently cured or for a long time without symptoms.

It would seem when we analyze the conditions in syphilis that complete sterilizing immunity or, in other words, complete cure, occurs but rarely without specific medicinal aid, and that the untreated syphilitic (if such an unfortunate individual exists in a civilized country) might go on to apparent cure, in that a general syphilization of his body would bring about a general resistance, but would always harbor virulent treponemata which could cause recrudescences in parts in which resistance was diminished, and eventually kill by degenerative processes in the central nervous system where many injuries cannot be compensated for as is possible in other organs.

The resistance which develops is apparently a new attribute only of the cell groups which have undergone direct reaction with the treponemata. This resistance may consist merely in the complete failure of the tissue cells to react to the virus, a sort of "tissue indifference" or "Anergie." It may be, however, and probably is, accompanied by a certain amount of active defense in the form of local phagocytosis of the treponemata by the fixed tissue cells.

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THE ESTIMATION OF SUGAR IN THE BLOOD BY THE LEWIS-BEN-EDICT METHOD. A GRAPH FOR OBTAINING DIRECTLY THE PERCENTAGE OF DEXTROSE*

BY LOUISE McDANELL, NEW HAVEN, CONN.

PUBLISHED less than two years ago, the method of Lewis and Benedict¹ for the determination of blood sugar has been very widely and successfully used. In this method, the red color due to the reduction of picric acid to picramic acid by dextrose, in an alkaline medium, is made the basis of a colorimetric determination. Two c.c. of blood, measured in an Ostwald pipette, are called for. Variations of the original method have been proposed by Pearce,² and by Myers and Bailey,³ the same amount of blood being employed in each instance; and by Epstein,⁴ who uses but a fraction of a cubic centimeter of blood.

It frequently happens, however, that it is more convenient to weigh the samples of blood than to measure them, and also that, owing to the delicacy of the method, when duplicate determinations are not essential, smaller samples are sufficient. Especially is this true when it is desirable to take a large number of samples from small animals.

The details of the method, as we have applied it, are here given.

Glass stoppered weighing bottles holding about 30 c.c. (22 by 75 mm.) are graduated to 12.5 c.c. About 3 c.c. of water are introduced into the bottle, and the whole accurately weighed. Into this about 1 c.c. of blood is allowed to drop from a small cut in the ear vein of the rabbit (as described later), care being taken to insure thorough hemolysis. The bottle is weighed at once. Seven and a half c.c. of a saturated aqueous solution of picric acid are added, the contents made up to the mark with water, and thoroughly shaken, thus precipitating completely the proteins of the blood. From this it is not difficult to obtain 8 c.c. of filtrate, if small folded filters are used, or better, hardened papers with the aid of suction. Eight c.c. of the filtrate are pipetted into a Jena glass test tube, at least 22 by 200 mm. in size, to which are added 2 c.c. of the saturated picric acid solution, 1 c.c. of a 10 per cent solution of sodium carbonate (anhydrous salt), 4 drops of kerosene (instead of mineral oil) to prevent frothing, and 2 or 3 glass beads. This solution is evaporated to dryness over a free flame. With the use of a very hot flame and constant shaking the evaporation may be accomplished in two or three minutes, without loss of material.

The residue is dissolved, with the aid of heat, in about 3 c.c. of water, transferred quantitatively to a small test tube, graduated to 10 c.c., and diluted to the mark. These test tubes have been found entirely satisfactory, and can be graduated much more easily than 10 c.c. volumetric flasks can be obtained at the present moment. Traces of kerosene that remain are removed by filtering, through small folded filters, into the colorimeter chamber or another test tube. This solution should stand not less than five nor more than twenty minutes before being compared in a Duboscq colorimeter with the standard described below.

*From the Sheffield Laboratory of Physiological Chemistry, Yale University.

Daylight from a north window is always used for reading the colorimeter, the mirror being set at the angle to give the lightest yellow tone to the standard. In testing out the method, this was found necessary in order to obtain uniform results. If it is late in the day, the material, evaporated to dryness, is left in the Jena glass tube until the next morning.

The standard color, with which the unknown solution is compared, is obtained from 0.64 mgm. of dextrose, 5 c.c. of saturated picric acid, and 1 c.c. of a 10 per cent solution of sodium carbonate, evaporated over a free flame, and

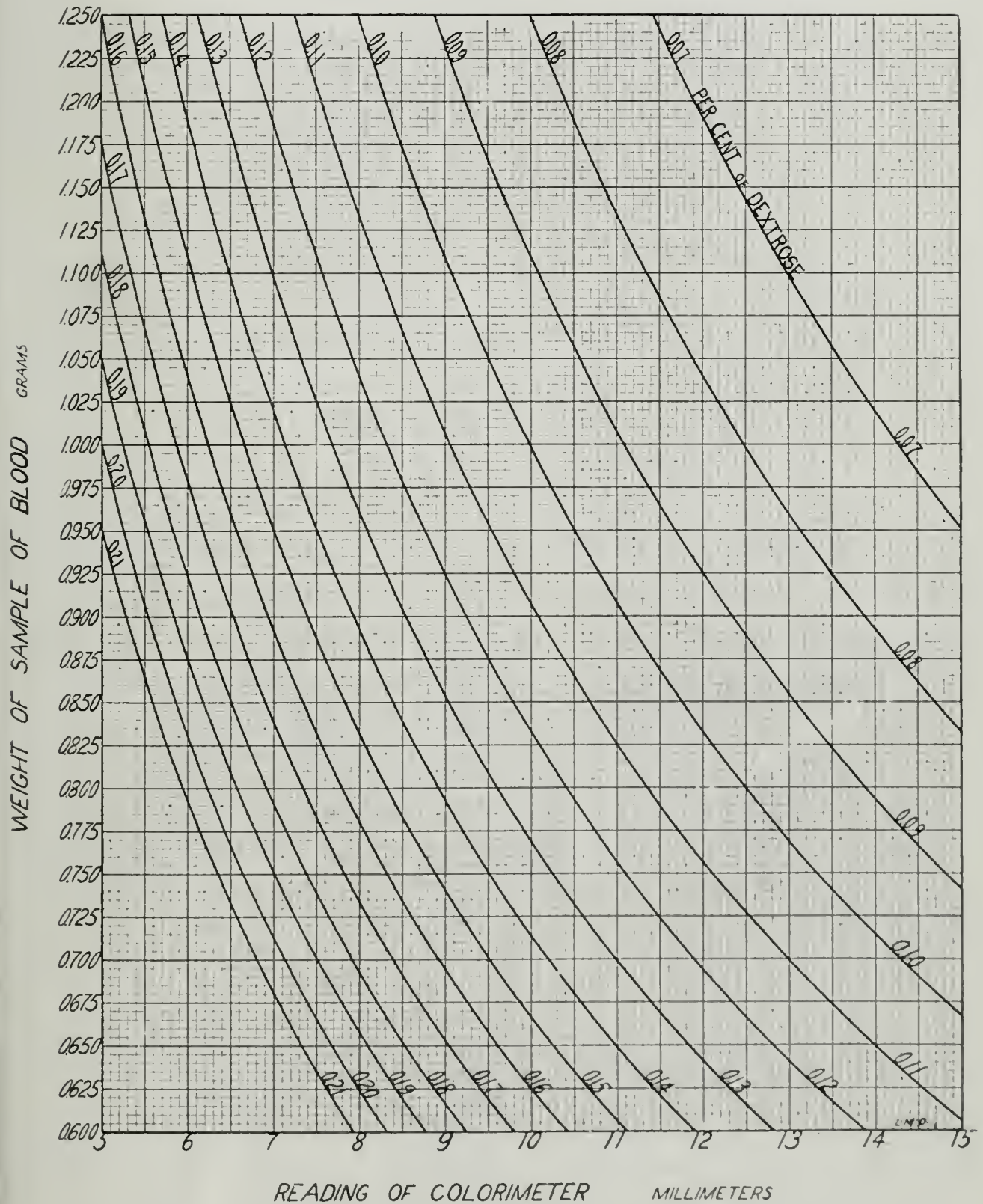


Fig. 1.—Graph for obtaining the percentage of dextrose in the blood (Lewis-Benedict method).

diluted to 10 c.c. as was the unknown. A satisfactory permanent standard consists of a solution of picramic acid, which has approximately the same color, and keeps perfectly. It requires 0.064 gm. of picramic acid and 0.100 gm. of anhydrous sodium carbonate. The picramic acid is dissolved, with the aid of heat, in 25-30 c.c. of distilled water made alkaline with the sodium carbonate. This is cooled and diluted to one liter. It should be standardized against pure dextrose.

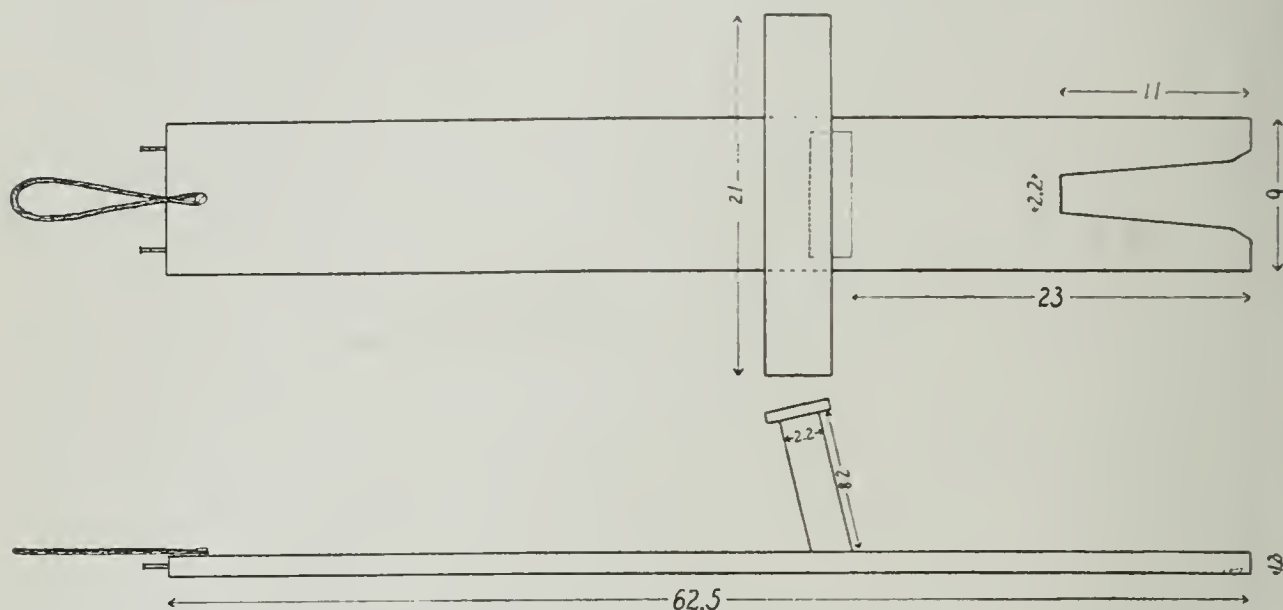


Fig. 2.—Diagram of "boot-jack" holder for rabbit. The dimensions are given in centimeters.



Fig. 3.—Rabbit fastened to board.

The general formula given by Lewis and Benedict, for the percentage of dextrose in the unknown is:

$$\text{Percentage of dextrose in blood} = \frac{\text{reading of standard}}{\text{reading of unknown}} \times \frac{\text{mgm. of dextrose in standard}}{\text{grams of blood used}} \times 0.1$$

Under the conditions here specified—the standard containing 0.64 mgm. of

dextrose, with the Duboscq colorimeter set at 10 mm., and the actual use of 8/12.5 of the sample taken—this formula becomes:

$$\text{Percentage of dextrose in blood} = \frac{1}{\text{reading of unknown} \times \text{weight of sample—grams}}$$

To avoid calculations, the accompanying graph (Fig. 1) was constructed. Weights of blood are represented by the ordinates, and colorimeter readings of the unknown by the abscissas. For a given sample, the relation of the point of intersection of these two lines to the percentage curves between which the point lies is readily determined. For example, if the sample of blood weighs 0.89 gms., and the reading of the colorimeter is 10.7 mm., the sample contains 0.105 per cent of dextrose. The points on the curves were calculated from the formula, which is used whenever either of the values lies beyond the range of the chart. As suggested by Lewis and Benedict, when the percentage of sugar in the blood is high, the final volume should be made 25 c.c. or 30 c.c., instead of 10 c.c., and the results, as given by the graph, multiplied by 2.5 or 5.0.

If it is desired to make duplicate determinations, approximately twice as much blood should be taken, 15 c.c. of picric acid added, and the volume made up to 25 c.c. Using 8 c.c. aliquots, as above, the results are readily obtained from the graph, if the weight of the sample is first divided by two. Similarly the graph is available for use with the original method in which 2 c.c. of blood are used, the value for the weight of the sample then always being 1 gram.

A METHOD FOR OBTAINING BLOOD FROM RABBITS.

The description of a method for collecting blood from rabbits, which has been in use successfully for several years in this laboratory may be a help to workers in other places. None of the devices employed present any features of extreme novelty, but the mode of their application appears not to be widely known.

The animal is fastened gently on the familiar "boot-jack" holder, which is shown in Fig. 2. Fig. 3 shows the animal in position on the board. When it is desired to take blood from the ear, the board is hung as shown in Fig. 4. After the fur is clipped from a portion of the ear over the vein to be used, preferably the marginal one, a small cut across the vein is made with a very sharp razor, and the blood is allowed to drop into a container. By this method varying amounts of blood may be obtained easily and quickly, and samples may be taken many times from the same animal. Recently we have taken samples from rabbits twice daily for six weeks.

The ear must be kept dry to prevent the blood from spreading on the surface, any clotting blood being removed with absorbent cotton. A good blood-flow may best be promoted by rubbing the ear vigorously before the animal is put on the board. The circulation may be further improved by bringing a lighted electric bulb near the ear. Toluene and xylene, widely recommended for use in producing local hyperemia, are too irritating for frequent application. Usually the bleeding may be stopped by pressing a small piece of cotton against the cut and holding it there a few seconds to allow the blood to clot; or an artery clamp may be used, though the clamp is avoided wherever possible, as

its use increases the difficulty of obtaining samples from the same place subsequently. Alcohol is used to disinfect the cut after the sample has been taken.

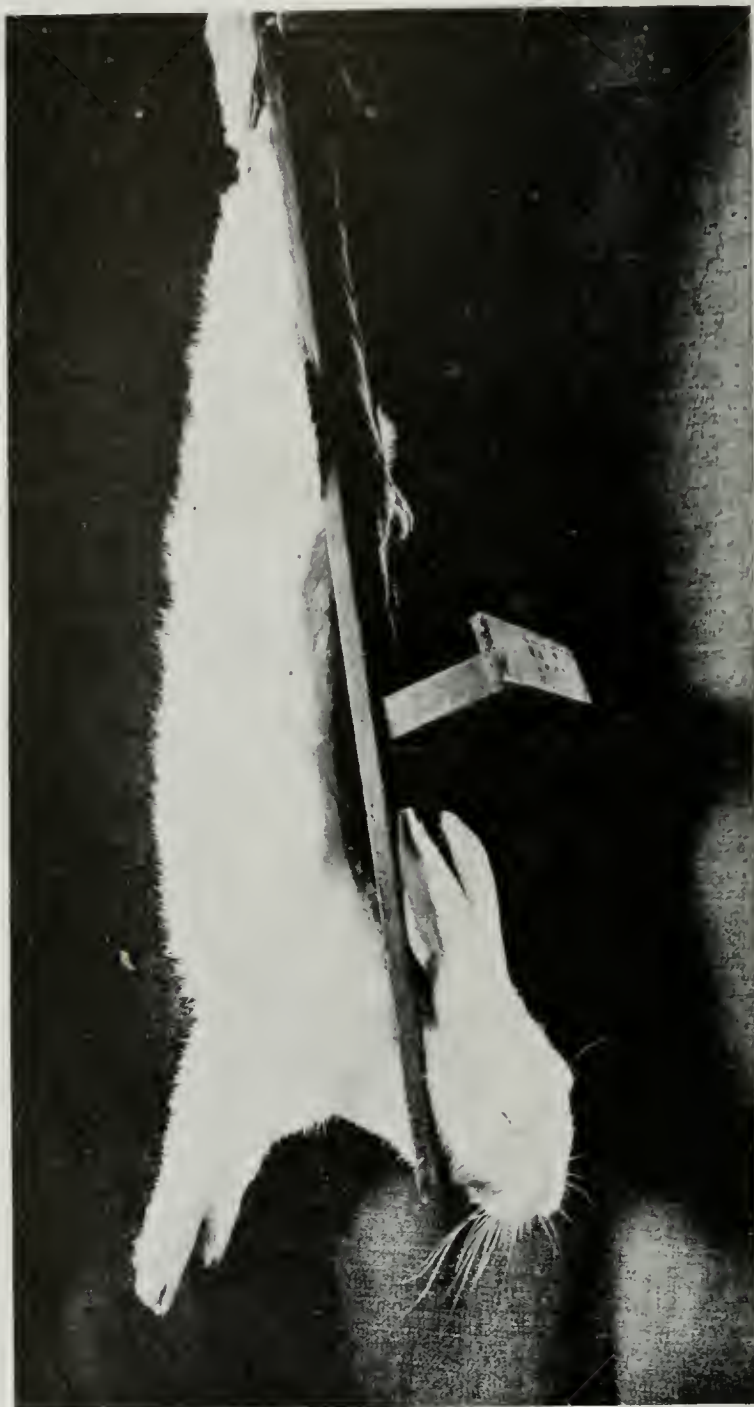


Fig. 4.—Rabbit in position for taking blood from ear.

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THE ROLE OF CHOLESTEROL IN PATHOLOGY*

BY J. C. SMALL, CHICAGO, ILL.

CHOLESTEROL occurs universally distributed in the plant and animal tissues, occurring in the former in an isometric form called phytocholesterol. Chemically it is classed in the sterol group of lipins. It was first isolated from gall stones in 1785 but its true, nonsaponifiable, fat-like nature was not discovered until 1814. When pure it crystallizes in white, plate-like rhombic crystals which are soluble in soap solutions; readily soluble in bile salts; and freely soluble in hot alcohol, hot or cold ether, carbon disulphide, chloroform, benzene and acetone. Its exact molecular structure is not known.¹ The following groups have been identified: a secondary alcohol standing between two methylene groups, a terminal vinyl group, and probably four saturated hexa-carbon rings. There is one, or perhaps two, double bonds. It belongs to the terpenes and is probably closely related to the bile acids. Its empirical formula is $C_{27}H_{45}OH$ or $C_{27}H_{43}OH$. In general its chemistry is that of secondary alcohols. It readily forms crystalline esters many of which on cooling show a beautiful play of colors.

Quantitatively, it may be determined gravimetrically, or colorimetrically by recently perfected methods.² The gravimetric method is based on the reaction between cholesterol and digitonin which results in the formation of a precipitate sufficiently stable to be dried and weighed. The colorimetric methods are based essentially on the high color property of various cholesterol esters, the color values developed in unknown solutions being read by means of a standard colorimeter against that developed in a known solution. Colorimetric methods are admirably adapted to a study of the fluids of the body, but at best they are less accurate, giving slightly lower results than the digitonin method.

The physiological significance of cholesterol has been the subject of much controversy. Even now our knowledge of this subject lags sadly behind the knowledge of its pathological significance. The work on which the present views of the metabolism of cholesterol are based has practically all been done by one group of English investigators.

Of historical interest is Flint's³ idea suggested in 1862 that cholesterol is an important factor in intoxications, especially in icterus. He claimed that the liver excreted cholesterol from the blood and that any disturbance of this function, resulting in bile retention, caused an accumulation of cholesterol in the blood with subsequent symptoms of toxemia.

Following him, Pages⁴ in 1896 and Müller⁵ in 1873, injected cholesterol into the blood stream with results which led them to confirm its toxic properties. Felz and Ritter,⁶ in 1876, and Rywasch,⁷ in 1888, repeated these experiments with opposite results establishing the view which has since been repeatedly confirmed, that cholesterol is non-toxic. In 1902 Kaiserling and Orgler⁸ described under the name of "myelin" certain intracellular droplets in the normal adrenal cortex which differed from fat in being doubly refractive and in staining but slightly

*From the Department of Pathology, University of Illinois.

gray with osmic acid. In 1907 Panzer⁹ and also Aschoff¹⁰ showed these to be droplets of cholesterol esters. About the same time Lorrain Smith¹¹ and Dietrich¹² separately devised methods for the differential staining of these droplets and a little later Aschoff¹³ and Kawamura¹⁴ presented the first detailed morphological studies of them.

With the perfection of these methods for the morphological and the chemical study of cholesterol the work received a great impetus. As previously mentioned, one group of investigators addressed themselves to the physiological side of the problem. This work appears under the authorship of Dorie, Ellis, Gardner and co-workers¹⁵ in a series of articles from 1908-1912 entitled "The Origin and Destiny of Cholesterol in the Animal Organism." Their earlier work suggested the following hypotheses:

1. Cholesterol is present in all cells, and it is not excreted as a waste product when the cell breaks down, but is resorbed and utilized in building new cells.
2. A function of the liver is to break down red blood cells and eliminate their cholesterol into the bile.
3. Cholesterol in the form of its esters is resorbed along with bile from the intestine.

Their later work supports these hypotheses and seems to warrant the following conclusions:

1. An increase in the cholesterol and cholesterol esters of the blood and liver directly proportional to an increased intake of cholesterol in the diet occurs in animal feeding experiments, but the kidney content is not affected.
2. Where food is withheld (inanition) there occurs an increase in the cholesterol content of the blood, liver and kidneys as well. In the latter the increase is due for the most part to cholesterol esters.
3. In the human a cholesterol balance can be established, i.e., the ingested cholesterol can all be accounted for in the feces, but the incidence of an acute infection with loss of weight disturbs this, yielding a positive balance in the feces.

Within the last year, Lander¹⁶ reports results which prompt him to emphasize the ability of the organism to pick out and conserve cholesterol from a diet of an exceedingly low cholesterol content. The normal content of the various tissues and fluids will be considered at another place insofar as it bears on the pathological conditions to be studied.

Qualitative considerations with reference to the cholesterol content of the blood and bile are of interest in view of the light which they shed upon a function of the liver. The cholesterol content of normal blood is fairly constant (1.5 to 1.8 gms. p.m.) and is so distributed that the cellular elements contain largely free cholesterol while the blood serum contains both free cholesterol and cholesterol esters. The corpuscle content is affected but little by enriching the medium in which they float. It has been pointed out that while the blood on the whole is richest in cholesterol esters, the bile is richest in free cholesterol and that an increased content of the former induces a corresponding increase in the latter.¹⁷ This would seem to suggest a specific enzyme for splitting cholesterol esters. Such a cholesterase has been described.¹⁸ The recognition of the occurrence of cholesterol in pathological conditions dates back to the time of its preparation

from gall stones. From the standpoint of the morphologist a vast number of conditions have been known. A few of the more important ones only can be considered.

1. The so-called "pure" cholesteral gall stones range from 90 to 98 per cent of pure cholesterol. Their formation has variously been attributed to decreased alkalinity, decreased amount of bile salts, inflammation, colloid deflocculation and to an increased secretion of cholesterol with resorption of its solvents from stagnating bile.¹⁹ The frequency of gall stones in women as shown by clinical and post mortem records²⁰ and the high cholesterol content of the bile during pregnancy are significant facts in view of the latter theory.

2. Cholesterol crystals occur in the tissues in a great variety of conditions, such as the atheromatous patches in blood vessels, encapsulated caseous areas, old infarcts and hematomas, dermoid cysts and, in especially large amounts, in the cholesteatomatous tumors of the ear and cranial cavity. LeCount,²¹ in 1902, was the first to describe accurately the cholesterol giant cells so frequently seen in these conditions. Crystals also occur in old pleural effusions, hydrocele fluids, and in the cerebrospinal fluids, especially in tertiary syphilis.

Kaiserling and Orgler⁸ on recognizing the anisotropic droplets in the normal adrenal cortex extended their studies to pathological conditions and identified them in amyloid kidneys, pneumonia exudates, retrogressive tumor and thymus changes, corpus luteum, etc. This condition has been called cholesterol ester fatty metamorphosis²² and is especially prone to occur in the parenchymal cells derived from the urogenital anlage. Most conspicuous deposits occur in the epithelium of the "large white kidney," which are identical to the "protagone" bodies of the earlier histologists. This has also been spoken of as the setting free of the invisible cell lipin by autolytic processes akin to those giving rise to the initial cloudy swelling of true fatty degeneration.²³ There is some evidence to show that in the case of some organs, at least, an additional factor this latter is of exogenous or of endogenous origin, i.e., from the autolysis of plays a part.²⁴ This is a hypertension of cholesterol in the blood stream whether other organs.

Broadly speaking, then, deposits of cholesterol esters occur pathologically at the sites of low grade chronic inflammation, while deposits of cholesterol crystals occur under conditions of slow cell destruction in areas where absorption is poor.

The quantitative changes in the lipin content of the blood serum under pathological conditions have been the subject of much study^{2, 25} from the standpoint of cholesterol and its esters.

Quantitative changes: (a) An increase of total cholesterol is found in arterial sclerosis, chronic Bright's disease, jaundice, diabetes mellitus, xanthoma, eclampsia, pregnancy, and obesity. (b) A decreased amount is found in pernicious anemia, all febrile diseases (except enteric fever), in tuberculosis, and in old age. The normal amount in the blood is placed at from 1.5 to 1.8 gms. p.m.

In arterial sclerosis associated with²⁶ chronic Bright's disease, there occurs from two to four grams p.m. while the content of the aortic wall is also greatly increased.²⁴

In diabetes mellitus the increase appears to vary directly with the degree of acidosis and may be placed at from 3 to 4 grams p.m.²⁷ In hepatic disease with jaundice there is an increase varying with the degree of the jaundice but on the whole of less marked proportions than in the above conditions.

The blood analysis in xanthoma not associated with the above conditions, gives high values.²⁸ The fact that 70 per cent of such cases occur in women and particularly at the menopause vaguely connects the etiology with ovarian disturbances.

The increased cholesterol content of the blood during pregnancy is constant.²⁹ It is most marked during the last month. Values ranging from 2.5 to 4.25 gms. p.m. are given. An increased elimination occurs during the puerperium, chiefly through the milk, the hypercholesterolemia passing off the more rapidly in the nursing mothers. The blood of the newborn child is low in cholesterol content (1.19 gms. p.m.). The bile and adrenal content appears to parallel the blood content in these conditions of hypertension.

No explanations are offered to account for the hypocholesterolemia occurring in the above mentioned conditions. The suggestion that the cholesterol is used up in combating acute infections in an immunity reaction seems unwarranted. The positive balance of cholesterol¹⁵ in the feces during acute infections and its increased storage in the reserve fat of old persons³⁰ deserve passing mention in this connection.

EXPERIMENTAL WORK WITH CHOLESTEROL.

Since the work of Ignatowski, in 1908, the question of the artificial production of artery lesions by excess feeding of certain foods has received attention.³¹ Cholesterol has been identified as a food constituent producing this change. In addition to atheroma, cirrhosis and possibly kidney lesions are accredited to increased cholesterol ingestion.⁸ So far such lesions have been produced only in rabbits, all experiments on carnivora being uniformly non-productive. It must also be added that atheroma and sclerosis are relatively frequent lesions in rabbits.

The relation of cholesterol to the growth of neoplasms has been studied in the case of experimental carcinomas in rats,³² with results which seem to indicate that it accelerates the rate of growth and that it may be a factor in the incidence of cancer. This fact and the observation of an increased cholesterol content in the reserve fat of old people³⁰ may furnish a basis for the explanation of the increased incidence of cancer past middle life. With the cholesterol content of the tissues directly influenced by the diet,¹⁵ in view of the preceding observations the question arises—can the incidence of cancer be decreased by decreasing the ingested cholesterol? Experiments have as yet failed to show this. In this connection, it is well to recall that the organism tends to accumulate and conserve cholesterol so that it is more difficult to decrease than it is to increase the cholesterol store of the tissues.¹⁶ Examinations of the cholesterol³³ content of carcinomas in rats show that it is not affected by cholesterol injections; that it increases with the age of the tumor; and that the outside parts of the tumor mass contain less than does the central portion.

The part which cholesterol plays in phagocytosis and in immunity reactions

is very interesting and at the same time but little understood. It is well known that alcohol, chloroform and ether are detrimental to the natural defences against disease. They suspend phagocytosis both without and within the organism. This action has been attributed to their solvent power for the cell lipins in view of the fact that the administration of lipins tends to offset this effect within the organism. The experimental work³⁴ undertaken to show the relation of lipins to phagocytosis has been productive of results varying from inhibition on the one hand to increased phagocytosis on the other hand. This variety of results undoubtedly has arisen because *lipin mixtures* were used, i.e., the ether soluble tissue components, containing both lecithin and cholesterol which in this connection are antagonistic. Cholesterol even in small quantities vigorously inhibits phagocytosis but this inhibition can be completely overcome by lecithin.³⁶

Under the heading of hemolysis, we must consider, separately, the actions of cholesterol and cholesterol esters. Cholesterol counteracts saponin hemolysis by chemically combining with the saponin, thus precipitating an inert cholesterol-saponin compound. In hemolysis by cobra venoms, cholesterol plays a somewhat different part. The hemolytic principle of cobra venom resembles an amoebocyte in that it is complemented with lecithin³⁵ while the hemolytic action of the substance thus produced is very great, it is very markedly inhibited by cholesterol. Two explanations⁴¹ are suggested. The action may be physical or chemical. The first assumes the formation of a definite compound, a "lecithid" which hydrolyzes and yields lecithin. This lecithin is absorbed and carried down by the cholesterol as the unstable colloidal solution of the latter deflocculates. The chemical explanation assumes the formation of a definite chemical compound between the lecithin hemolysin combination and the cholesterol. Whether or not cholesterol plays a similar role in counteracting hemolytic bacterial toxins within the organism is not known. The question is of vital importance in all conditions associated with hemolysis. The administration of cholesterol is accredited with improving some cases of pernicious anemia.

Cholesterol esters do not inhibit cobra venom hemolysis.⁴¹ There is evidence to show that cholesterol esters of unsaturated fatty acids may be a factor in furnishing complement for the cobra venom hemolysis.³⁵ These esters themselves may be directly or indirectly hemolytic. *Bothriocephalus* anemia is attributed to the cholesterol ester of oleic acid.³⁷ Various investigators have shown the hemolytic properties of unsaturated fatty acids.³⁸ In phosphorus and also toluylendiamin poisoning fatty acids have been demonstrated in salt extracts of the liver.³⁹ There is some dispute as to the efficiency of fatty acids as hemolytic agents. McPhedron³⁸ claims that the hemolytic activity is not determined by the degree of saturation. Lomar³⁸ believes differently and King³⁸ supports him.

In a series of clinical conditions associated with hemolysis, King studied the unsaturated fatty acid and the total cholesterol of the blood. He found an increase in the former and a decrease in the cholesterol. He connects this condition with hyperfunction of the spleen. He believes that this organ regulates the unsaturated fatty acid content of the blood. Its removal lowers the fatty acid concentration and raises the antihemolytic substances, i.e., total fat and cholesterol. On this basis then is explained the difficult hemolysis following

splenectomy as well as the reputed improvement in cases of pernicious anemia thus treated.⁴⁷ The striking resemblance in the clinical picture of severe bothriocephalus anemia and pernicious anemia should be recalled. On the other hand in simple anemia no such variations in cholesterol and fatty acid content occur. There seems to be a growing tendency to associate hemolytic lipins with the etiology of pernicious anemia.⁴³

A striking example of endogenous hemolytic lipins is found in the "red degeneration" of uterine fibroids.⁴⁴ The amount of lipin in the ether extract of such fibroids varies directly with the degree of degeneration. From these tumors an active hemolytic agent has been isolated the action of which seems to be antagonized by normal serum. An excess of this hemolytic lipin acting on corpuscles in vitro produces first hemolysis without hemoglobin color changes, but later a brown precipitate settles, leaving a perfectly clean supernatant liquid. These results recall the physical explanation of the action of cholesterol in cobra venom hemolysis and suggest the spontaneous development of an endogenous lysin in retrograd focal tissue changes which not only affects the red cell stroma but also the cell proteins as is indicated by the color change produced in the hemoglobin.

The striking analogy between the inhibiting influence of cholesterol on the lecithin complemented cobra venom hemolysin, and the action of nonspecific antigen in complement fixation is apparent. Especially significant in this connection, are the recent observations which rank cholesterol enriched alcoholic tissue extracts as the most efficient antigen in the Wassermann reaction. Noguchi and Bronfenbrenner⁴⁰ contend that the antigenic value of the acetone insoluble fraction of the tissue extracts varies almost directly with its power to combine with iodine, i.e., with the amount of unsaturated fatty acids present.

Browning et al⁴¹ show that cholesterol is more efficient than any of its compounds in enriching the alcoholic extracts for use as antigen. Walker and Swift⁴² go still further in concluding that the cholesterol enriched alcoholic extract of heart muscle fulfills the requirements of a standard antigen in the syphilis reaction. With regard to the substances in the syphilitic sera which participate in the Wassermann reaction, it may be stated that while there appears to be a lipin increase in syphilitic sera,⁴⁵ no relation can be established between the lipin content and the antibody titer.⁴⁶ On the whole, the heterogeneous nature of the lipins taking part in this reaction prevents any conclusions as to the exact action of cholesterol.

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A REVIEW OF THE COMPLEMENT FIXATION TEST IN TUBERCULOSIS*

BY H. R. MILLER, M.D., NEW YORK CITY.

FOR a great many years laboratory workers interested in tuberculosis have attempted to perfect methods of complement fixation for the diagnosis of this disease. The earlier work upon this subject was not a little discouraging because the results obtained were not uniform and were often out of harmony with the clinical analysis of the cases. Fixation reactions, therefore, had much laboratory interest but were of little value to the physician.

More recent investigations have been more encouraging and methods have been reported from a number of laboratories by which results sufficient to demonstrate the formation of circulating antibodies and often of great diagnostic value, have been obtained.

For the detection merely of the existence of tuberculosis in a patient, the physician has had at his disposal not only accurate clinical methods, but tuberculin tests of great delicacy. By complement fixation reactions it was hoped not only to establish additional diagnostic data, but by measuring the fluctuations of antibodies in the circulating blood, to throw light upon the activity of the existing disease and obtain prognostic information.

This review attempts to show how successfully this aim has been accomplished and along which lines of attack complement fixation in this disease has been planned and carried out.

From a study of the literature on the subject, it is clear that the principal difficulty consisted in procuring proper antigens. In the main, the general methods employed varied but little from the technic of the Wassermann reaction. The antigens however differed greatly and were as numerous as the different workers who devised and employed them. Almost all the tried antigens fall into four general groups.

Group I. Antigens in which the whole tubercle bacillus was used. This group includes antigens prepared from all the tubercle bacillus strains as well as from the allied acid-fast bacilli, i.e., smegma, butter bacillus (Rabinowitsch), bacillus lepræ, Grassberger's milk bacillus, and the Timothy hay bacillus. The bacteria were used in suspensions, living, and, after treatment, heat, cold, etc.

Group II. These antigens consisted of tuberculins. Practically all tuberculins have been made use of as antigens. Some have yielded good results, and in the hands of a limited number of writers the reports have appeared to be of great value.

Group III. This group comprises a large series of antigens which may be termed "tubercle bacillus derivatives or extracts." They represent many preparations obtained by subjecting the bacilli to various processes of extraction and digestion. The "partial antigens" of Much and Von Deycke are of this kind.

Group IV includes the antigens produced from normal and from tuberculous tissues, i.e., lung, kidney, brain, lymph-nodes, spleen.

*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

The following group tables indicate, though not completely, the number of antigens that have been employed:

GROUP I.—BACILLARY SUSPENSIONS.

NAME.	ANTIGEN.	RESULTS.									
Caulfield and Beatty: Jour. Med. Research, 1911, xxiv, 122.	Bacillary emulsion.	Stage I.—33% positive. Stage II.—70% positive. Stage III.—62% positive. No fixation in normal cases.									
Laird: Jour. Med. Research, 1912, xxvii, 163.	Watery emulsion.	4 positive out of 34 tuberculous patients.									
Calmette and Massol: Compt. rend. Soc. de biol., 1912, lxxiii, 122.	Killed and concentrated bovine emulsions.	Results uncertain.									
Fraser: Ztschr. f. Immunit., 1914, xx, 291.	Bacillary emulsion.	<table> <tr> <td></td><td>Posi- tive.</td><td>Nega- tive.</td></tr> <tr> <td>Tuberculous sera</td><td>1</td><td>12</td></tr> <tr> <td>Nontuberculous sera</td><td>4</td><td>16</td></tr> </table> <p>Luetic sera may give positive reaction.</p>		Posi- tive.	Nega- tive.	Tuberculous sera	1	12	Nontuberculous sera	4	16
	Posi- tive.	Nega- tive.									
Tuberculous sera	1	12									
Nontuberculous sera	4	16									
Schultz: Ztschr. f. Immunit., 1910, vi, 709.	Bacillary emulsion.	Fixation in 5 out of 34 tuberculous cases.									
Bang and Anderson: Centralbl. f. Bakteriöl., orig., 1913, lxi, 517.	Bacillary emulsion, killed.	Reaction strong in positive cases; varies with severity of the infection. Tested cattle only.									
Zweig: Berl. klin. Wchnschr., 1912, xlix, 1845.	Bacillary emulsion.	Fixation proportional to amount of infection. (Used active sera.)									
McIntosh and Fildes: Lancet, London, 1914, ii, No. 8, 485.	Living fresh emulsions.	Pulmonary tuberculosis 76.7% positive. Surgical tuberculosis 80.7% positive. Syphilitic sera not fixed.									
Radcliffe: Ibid., 488; Jour. Hyg., July, 1915, 36.	Bacillary emulsion living and freshly prepared.	Sputa positive pulmonary cases: Stage I 88.6%. Stage II 89.6%. Stage III 79.0%. Controls negative.									
Loeffler: Deutsch. med. Wchnschr., May, 1913, 1025.	Bacillary emulsion, heated, dry.	Uncertain.									
Stimson: Bull. Hyg. Lab. U. S. P. H. and M.-H. S., No 101, Aug., 1915.	Killed and living bacillary emulsions.	Maximum fixation appears in later stages. Too few cases tested.									
Harris and Lanford: Jour. Infect. Dis., 1913, xiii, 301.	Suspensions of various acid-fast bacilli.	Demonstrated group fixing bodies, not specific for strains used.									
Miller and Zinsser: Am. Jour. Med. Sc. (in press), Proc. Soc. Exper. Biol. and Med., 1916, xxiii, 134.	Killed or living, salt triturated.										

GROUP II.—TUBERCULINS OR FILTRATES.

NAME.	ANTIGEN.	RESULTS.
Ruppel and Rickman: Ztschr. f. Immunit., 1910, vi, 344.	Old tuberculin.	Fixation only in tubercular animals.
Armand, Delille, Rist and Vaucher: Compt. rend. Soc. de biol., 1913, lxxiv, 791.	Crude tuberculin.	100 cases tested. 4% positive fixation. 30% partial fixation.
Davidovics: Deutsch. med. Wchnschr., 1914, xl, 1, 21.	Old tuberculin.	Results uncertain; used active sera.
Besredka: Ztschr. f. Immunit., 1914.	Besredka tuberculin.	Stage I. Reaction always positive. Stage II. Reaction nearly always positive. Stage III. Reaction partial or negative. Luetic sera not infrequently fixed. Normal sera occasionally fixed.
Debains and Jupille: Compt. rend. Soc. de biol., 1914, lxxvi, 199. Ann. de l'Inst. Past., Apr., 1915.	"	Stage I and II (fair condition) 90.3% positive. Stage II and III (bad condition) 81.3% positive. 24% positive Wassermann sera gave positive tuberculosis fixation. Nontuberculous sera 17.3% positive. Normal sera 3.2% positive.
Kuss, Leredde and Rubinstein: Compt. rend. Soc. de biol., 1914, lxxvi, 244.	"	Positive in 89% of developed pulmonary tuberculosis. Not always negative with luetic sera.
Inman: Compt. rend. Soc. de biol., 1914, lxxvi, 251.	"	100 tuberculous sera tested; 95 gave positive reactions.
Bronfenbrenner: Arch. Int. Med., 1914, No. 6, 786. Ztschr. f. Immunit., 1914, xxiii, 2, 21.	"	Positive cases gave positive fixation 93.8%. Luetic sera gave positive 43%. Nontubercular sera gave positive in 18 out of 220 cases.
Renaux: Compt. rend. Soc. de biol., 1914, 864.	Besredka tuberculin, extracted with ether.	Avoids nonspecific fixation of luetic sera.
Petroff: (Not yet published.)	Petroff's tuberculin, potato broth.	High number of fixation in positive cases. Not infrequent fixation with luetic or inactive sera.
Craig: Am. Jour. Med. Sc., Dec., 1915, 781.	Besredka tuberculin, modified, alcoholic extraction.	Active tuberculosis fixation in 96.2%. Inactive tuberculosis fixation in 66.1%. Not positive in nontuberculous and luetic sera.

GROUP III.—BACILLARY EXTRACTS.

NAME.	ANTIGEN.	RESULTS.
Borissjak, Sieber and Metalinkov: Ztschr. f. Immunit., 1910, xii, 65.	Products of tubercle bacilli.	Antibodies are not specific for the antigens administered.
Dielman: Ztschr. f. Immunit., 1911, x, 421.	"Partial antigens."	Fixation in clinically nontuberculous cases.
Calmette and Massol: Compt. rend. Soc. de biol., 1912, lxxiii, 120.	A. Exobacillary dialysed products. B. Endobacillary products. 1. Water soluble. 2. Peptone water soluble.	Greatest number fixation with B-2.
Much: München. med. Wchnschr., 1912, lix, 685.	"Partial antigens."	Fixation in 77% of healthy people.
Kurt Meyer: Ztschr. f. Immunit., 1912, xlv, 359.	Deprived products of tubercle bacilli.	Fixation by the phosphatid derivatives.
Rothe, Bierbaum: Deutsch. med. Wchnschr., 1913, 644.	Watery extract of bacilli.	Strong fixation in a small percentage of tuberculous cattle.
Dudgeon, Meek and Weir: Jour. Hyg., 1914, No. 1, 72.	Alcoholic extract.	Maximum fixation in moderately advanced cases.
Stimson: Bull. Hyg. Lab. U. S. P. H. and M.-H. S., No. 101, Aug., 1915.	Calmette's B-2.	In 234 positive pulmonary cases 89% positive. Negative reaction is of little value.

GROUP IV.—TISSUE ANTIGENS.

NAME.	ANTIGEN.	RESULTS.
Hammer: Deutsch. Tierarz. Wchnschr., 1912, xx, 593. Münch. med. Wchnschr., 1912, lix, 1750.	Old tuberculin with extracts of tuberculous nodules.	43 positive reactions out of 46 tuberculosis cases.
Bierbaum and Berdel: Ztschr. f. Immunit., 1914, 21, 1-5, 249.	Bovine old tuberculin and extracts of tuberculous tissue.	Autopsy findings and sera reaction agreed in 65% of cases.
Wolff-Eisner: Wien. klin. Wchnschr., xxxvii, 1908, 1296.	Ground-up tuberculous lung tissue.	Results uncertain.

Considering the data compiled above, we observe that, except for Hammer's work, there is little to commend in the antigens of Group IV, and it may be contended that the tuberculin and not the tissues employed may have been responsible for whatever apparent value may be attributed to Hammer's antigen.

The antigens in Group III at one time occupied the attention of many

workers. It was maintained that to yield available antigen, the tubercle bacilli must be subjected to processes of chemical extraction. Von Deycke and Much, and also Much alone, described various split products of the bacillus, obtained after prolonged extraction with fat solvents. They obtained specific antigens from the bacillus, and they held that these specific antigens were contained in both the fatty acids and protein of the organisms. Their researches with these specific "partial antigens" failed however to bring about any particular advance in the usefulness of the fixation reaction. Kurt Meyer's extensive studies in producing tubercle bacillus derivatives, the watery extracts of Dudgeon and others, the peptone extracts of Calmette and Massol, the fermentation and autolytic bacterial products—all these types of bacillary extracts were employed with varying degrees of success.

It was hoped that the antigenic properties of the tubercle bacillus were contained in a particular chemical fraction of the bacterial body and that it would be possible to obtain this separately. Their experiments were formulated in the endeavor to obtain such an extract and prove its antigenic properties by injecting it into animals and demonstrating specific antibody formation in the animal serum by fixation against the original extract. These expectations have not been fulfilled.

In Group II we are dealing with tuberculins. As early as 1903 Bordet and Gengou¹ demonstrated fixation of complement with Koch's O.T. Soon Wassermann and Bruck,² then Citron³ amplified these results in a limited series of cases. The many therapeutic tuberculins were investigated as antigens, and in addition numerous new tuberculins were proposed and tested.

Of recent years much attention has been focused upon the tuberculin described by Besredka. This is, briefly, a filtrate of an egg-meat-broth medium upon which tubercle bacilli are allowed to grow for several weeks. The filtrate only is employed as antigen. Good results with this antigen have been published by Besredka himself. Inman, Debains and Jupille have confirmed his findings. Renaux, Bronfenbrenner, and recently Craig have modified this antigen by extracting its lipins, and in this way seeking to avoid non-specific cross fixation with syphilitic sera. At best, however, the Besredka antigen shows a considerable margin of error. Craig, who reported no cross fixation and no fixation of non-tuberculous sera, obtained positive fixation in two-thirds of his clinically inactive cases, while Bronfenbrenner,⁴ in his last publication obtained complete binding with the Besredka antigen in 20 per cent of syphilitic sera, and in a number of patients who had no tuberculosis, the reaction was positive.

More recently still, Petroff at Saranac Lake obtained good results by using for his antigen a tuberculin made by growing tubercle bacilli upon a watery extract of potato to which glycerine had been added. Here, too, there was not infrequently non-specific fixation with syphilitic sera.

The antigens in Group III then, have been in general available for the fixation test, but the reaction still remained of limited help to the individual practitioner because of its errors in fixing, occasionally, the sera of normal, non-tuberculous and luetic patients.

For final consideration there remain the antigens in Group I. These consist of tubercle bacillus emulsions or suspensions. McIntosh and Fildes, Dudgeon,

Meek and Weir, and Radcliffe, used saline suspensions of living or killed bacilli. Radcliffe, particularly, published strikingly good results emphasizing the advisability of employing young and fresh cultures. Courmont and Arloing devised defatted emulsions. Harris and Lanford tried suspensions of a variety of acid-fast bacilli related to the tubercle bacillus. In spite of much that has been written about the anticomplementary effects of the bacillary emulsions used as antigens, an impressive number of laboratory workers has encountered little if any difficulty in this regard. The English workers especially have found these suspensions very satisfactory. They claim a high proportion of positive cases, but Radcliffe reports that 74 per cent of clinically arrested cases also gave binding.

Debré and Paraff⁵ proposed using tubercular exudates and transudates as antigen, titrating their antigenic properties against known, pooled tuberculous sera.

Recently Miller and Zinsser have employed an antigen prepared by triturating living or dead bacilli with dry crystals of ordinary table salt, then adding distilled water up to isotonicity. With this antigen the results have been as follows:

Of 232 clinically active cases with positive sputa, all but three gave positive fixation. One had an advanced pulmonary lesion; the disease in the other two was moderately advanced. Of ninety inactive or healed cases, eighty-three were negative. The reaction was positive in seven patients; five in this series had tubercle bacilli in their sputum shortly before the test was performed.* One hundred and forty cases were tested where the diagnosis was doubtful. In thirty-two the reaction was positive, and in some of these thirty-two cases the diagnosis of tuberculosis was subsequently established. Forty-three positive Wassermann cases gave no fixation with the tubercle bacillus antigen. Two cases were fixed with both antigens. In one the diagnosis of lues and tuberculosis was proved, the other case at present cannot be reached for investigation. One hundred and three non-tubercular cases were all negative.

From this rapid survey it will be seen that the bacillary emulsions, the tuberculins, and the extracts of bacilli, have yielded available antigens.

The filtrates of Besredka and of Petroff and the bacillary emulsion of Radcliffe, McIntosh have thus far served as the most available antigens.

The important factor has been established that in the fixation test we possess a method which will indicate for us the activity of tuberculous disease. The cutaneous skin reactions denote only a hypersusceptibility to the tubercle bacillus, and this state may persist even when the lesion is quiescent or healed.

With our own antigen the complement fixation in tuberculosis shows a remarkable degree of uniformity, in that practically all positive cases give positive fixation, and inactive cases are as a rule negative. Normal sera are always negative, and cross fixation with luetic sera is eliminated. The complement fixation test with this antigen can therefore serve as a measure for the control of the diagnosis of tuberculosis.

It seems that fair results have been obtained by a variety of methods. The

*Since the last published report (*Proc. Soc. Exper. Biol. and Med.*, 1916, xiii, 136) we have learned from subsequent data that some of the cases with positive sputum classed as inactive, belong to the active group.

writer believes from his own personal experience that the antigen of Miller and Zinsser is at present superior to the others in use because it has so far failed to give cross fixation with luetic sera, has usually been negative in arrested cases, and has been almost invariably positive with active cases. It seems also to be the one most easily prepared.

However this may be, and the eventual decision can come only with great multiplication of comparative data, it appears from the many records, both our own and those reported by many of the workers cited above that complement fixation in tuberculosis is a definitely valuable diagnostic method which will occupy an important place among the practical methods of diagnosis.

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THE QUANTITATIVE ESTIMATION OF PHENOL-TETRACHLOR-PHTHALEIN EXCRETED IN THE FRESH BILE IN DISEASE OF THE LIVER*

BY H. L. McNEIL, M.D., GALVESTON, TEXAS.

IT was first shown by Whipple and Rountree that phenol-tetrachlor-phthalein injected intravenously is not excreted in any appreciable amount by the kidneys, as is phenol-sulphon-phthalein, but is excreted largely by the liver.^{1, 2, 3, 4} It was shown, moreover, by Whipple, in experiments on dogs that artificial injury to the liver parenchyma, by chloroform or phosphorus poisoning, for instance, is accompanied by a marked diminution in the phenol-tetrachlor-phthalein output through the liver. Using the above preliminary experiments then as a basis for their work, Rountree and others in the same clinic concluded that by collecting all stools passed for forty-eight hours after an intravenous injection of 0.4 gm. of the dye, a fairly constant level of excretion for normal individuals was obtained, and that, moreover, the output was decidedly diminished in certain individuals suffering from disease of the liver. They concluded, therefore, that the test was of some value in diagnosis of such conditions.^{2, 5, 6, 7}

The conclusions of Rountree and his co-workers as to the value of their test in diagnosis have not, however, been substantiated by others. Sisson, Frazier and Kana all reported it as useless in diagnosis.^{8, 9, 10} As a result, therefore, of these contradictory reports, as well as of the fact that the technic of their test is rather cumbersome and time consuming, the test of Rountree and Whipple has been practically discarded.

During the course of certain work in which we have been engaged, we have had occasion to study a fairly large number of cases of cirrhosis of the liver. It

*From the Medical Department of the University of Texas.

occurred to us during this work that if after the injection of the phenol-tetrachlor-phthalein intravenously, we inserted a duodenal tube through the pylorus and, collecting all of the duodenal contents for a period of time by gentle aspiration, tested quantitatively for the dye which had been excreted in the bile, we might possibly obtain some information as to the excretory ability of the liver. Accordingly, a series of normal cases were first studied in this manner, the 0.4 gm. of phenol-tetrachlor-phthalein being injected according to the method described by Rountree and the contents of the duodenum being obtained during the ensuing two hours by continuous drainage. We hope that in this way two facts regarding the excretion of phenolphthalein could be ascertained: First, the time required for the first appearance of the dye in the bile, and, second, the quantity excreted in the bile during the two hours following the injection.

As will be noted from the following tables, the time required for the first appearance of the dye, after the intravenous injection, in normal individuals, varies from twelve to twenty-one minutes, while the amount excreted during the two hours following the injection varies from a trace to ten per cent of the amount injected. Moreover, it was found that the greater part of the two hour excretion occurred during the first hour after the injection, in normal cases.

After ascertaining the above facts regarding our normal cases, we have tried out the test, using the same technic upon a series of patients suffering from disease of the liver. The results of these tests were, briefly, somewhat as follows: Of five cases of atrophic cirrhosis of the liver (Laennec's cirrhosis) in well advanced stages, which were studied, the time required for the first appearance of the dye in the bile was somewhat extended as compared with the normal cases, varying in each case from twenty-eight to forty-five minutes, as compared with the time of from twelve to twenty-one minutes for the normal cases. The total two hour amount of bile collected showed, however, only slightly different percentages from the normal cases, three cases showing a practically normal output and only two varying somewhat from the normal, in that practically no dye was excreted in the bile which was recovered within the two hours. It was noted though, in those cases in which appreciable amounts of the dye were excreted, that practically all of the excretion occurred during the second hour as distinguished from the six normal cases in which most of the excretion occurred during the first hour.

In addition to the six cases of true cirrhosis, two cases of chronic passive congestion of the liver were studied in this way. None of these varied appreciably from the normal cases studied, however.

One case of extensive carcinomatosis of the liver was studied. In this case the initial excretion was decidedly delayed and practically none of the dye was excreted during the two hour interval, only a trace being found during the second hour.

This modification of the test has the advantage over the previous tests described by the originators of the method, that the dye is very easily estimated quantitatively in the duodenal contents, if the estimation is done promptly at the end of the collecting periods, the estimation being carried out in much the same way as in the kidney function test. There are several difficulties, however, which are met with in carrying out this test. One of these is that since the ejection of bile into the duodenum is apparently dependent upon the contractions of

the gall bladder, in the case of a supposedly delayed excretion time one may wonder if this is not due, perhaps, to a failure of the gall bladder to contract rather than to a really delayed excretion by the liver. Fortunately, however, there are certain simple procedures by which the contractions of the gall bladder can be, in a measure, controlled. One of the simplest of these, which we have found efficacious, is a simple change in the patient's position; from the side to the back, for instance. Repeated deep respirations associated with massage of the abdomen just under the right costal margin; the application of cold to the abdomen under the right costal margin; the causing of the patient to sit for a minute or so and then lie down again, will all often start up a sluggish gall bladder. Finally, the injection directly into the duodenum of from thirty to fifty cubic centimeters of a 0.5 per cent solution of hydrochloric acid^{11, 12} will occasionally start contractions of a sluggish gall bladder.

Still another difficulty met with in the attempt to obtain constant results in these cases, is the fact that fear, nervousness and hunger seem to have a more or less marked effect upon the secretion of bile. A perfectly normal case may, for instance, on the first application of this test, excrete only a trace of bile during the whole two hours. This is usually, apparently, brought about by fear, as is shown by the fact that the subsequent tests will show a normal excretion both of bile and of phenol-phthalein. Such a failure as this happened to us in two cases, in both, however, subsequent tests were quite normal. Recently, we have hit upon a method which counteracts in great measure this very serious difficulty. This is the allowing of a fairly liberal diet to the patient for a few days preceding the test and at the same time the administration of sodium bromide in fairly large doses, and finally and most important, the administration of sodium salicylate for its chologogic effect for several days preceding the test. Our usual dose of the salicylate is fifteen grains three times daily for three

DISEASE.	AMOUNT INJECTED.	1ST HOUR.	2ND HOUR.	TOTAL.	TIME OF APPEARANCE.
1. Normal	0.4 gm.	4%	6%	10%	20 minutes
2. "	0.2 gm.	Trace	6%	6%	25 "
3. "	0.5 gm.	4%	1%	5%	20 "
4. "	0.4 gm.	5%	Trace	5%	15 "
5. "	0.4 gm.	4%	"	4%	18 "
*6. "	0.4 gm.	Trace	"	Trace	21 "
*7. "	0.4 gm.	2%	4%	6%	12 "
8. Cirrhosis of Liver	0.4 gm.	Trace	3%	3%	30 "
9. "	0.4 gm.	"	5%	5%	28 "
10. "	0.4 gm.	"	9%	9%	45 "
11. "	0.4 gm.	"	8%	8%	35 "
12. "	0.4 gm.	"	4%	4%	32 "
13. Chronic Pas. Cong.	0.4 gm.	2%	2%	4%	20 "
14. "	0.4 gm.	4%	3%	7%	15 "
15. Carcinoma of Liver	0.4 gm.	Trace	Trace	Trace	35 "

*Numbers 6 and 7 represent tests done upon the same individuals, No. 7 being the second attempt. The discrepancy in the results is probably due to the fact that the patient was considerably frightened on the first attempt, by the manipulations incident to the test.

days before the test is carried out. The difference in the amount of bile excreted by those patients who have had a preliminary dose of salicylate and those who have not is often quite striking.

Finally, as a result of this limited number of tests, we believe that the quantitative estimation of phenolphthalein in the duodenal contents is of little value from the point of view of the information derived, but that the excretion time of phenolphthalein after its intravenous injection is rather decidedly delayed in certain pathologic conditions of the liver.

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FOCAL INFECTION*

BY JOHN W. SHUMAN, M.D., SIOUX CITY, IA.

THE subject is one with which the general practitioner is constantly confronted; it is a subject which has gained considerable prestige among medical men during the past decade. Many of you have witnessed the quick subsidence of clinical symptoms, the speedy and sure cure of disease, in a case of so-called "rheumatism" following the removal of the cause—the focus of infection.

Although focal infection is not a new discovery, it is often newly discovered in a patient who has suffered an ailment for weeks, months or even years due to the lack of early suspicioning and recognition of the focus of infection by the physician. For many years John Ridlon advocated that many cases of chronic joint disease were not tuberculous joints. He states (personal communication) that "The first case I recall in this connection was a knee joint inflammation associated with and apparently dependent upon an attack of dysentery, in September, 1882. I immobilized the joint and cured the dysentery and the case rapidly recovered." At that time little was known of gonorrheal joint disease in its acute and chronic forms, the same was true of syphilitic joint disease.

We must examine the patient minutely from "hair to toe nails," overlooking nothing which will be of aid in diagnosing the etiological factor. Focal infection gives rise to many symptoms. Its main feature is pain of nerve, muscle, or joint; one, two or all three. This generally brings the patient to the doctor for relief. If the disease is acute a part or all of the cardinal signs and

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symptoms of inflammation about one or more joints may be present (polyarthritis). An increase of temperature, pulse and white blood cells may be noted. Deep abscesses of the teeth are a common cause and often are overlooked for the simple reason that they are not suspected. I do not wish to tire you with case reports, but here is a brief report which speaks for itself.

C. J., Dentist. Examined Jan. 3, 1916. Complained of morning frontal headaches. Lost 16 lbs. in four weeks. Acute and painful swelling of the right heel and stiffening of the left knee. T. 99, 2. P. 97. W. B. C. 8,000. B. P. (s) 180, (d) 115-20, mm. Hg. Left heart border well outside the M. C. L. A systolic murmur transmitted to the axilla was present. Urine; alkaline, 1010, albumin a trace and two granular casts present. The second upper bicuspid root was abscessed as also was the second upper bicuspid (right). They were extracted and two weeks later he had no headaches. B. P. (s) 148 (d) 100 and no systolic murmur. Urine acid, 1022, no albumin or casts. A month later B. P. (s) 145 (d) 95. I called him in yesterday and confirmed the cardio-nephritic condition. This case is but one of many which I have had the privilege to observe in which the kidneys were protesting in such a manner that a man would have to be very careless in order to overlook.

The tonsil as a disease focus is well understood. Too well perhaps, for the first thing a patient with a pain in a muscle, nerve or joint, is advised, even by his friends, is to have the tonsils removed. Therefore many an unoffending tonsil is wantonly sacrificed.

One point in this connection I wish you to consider is the advisability of removing the acutely inflamed tonsil, the focus of infection. If this is done in the instance necessitating such a procedure, prolonged treatment, chronicity of disease and complications will be avoided. The abdominal surgeon once advised "to wait" concerning acute appendicitis, if pus was a factor, until the pus had walled off or the attack was over before operating. Now all agree that the sooner and the more thorough the appendectomy the better it is for all parties concerned. I believed this to be the rational treatment of focal infection elsewhere situated.

Thorough chest and abdominal examination is demanded in every instance. In order that this may be done properly the patient should be stripped, and not examined through the clothing. It is difficult enough to ascertain correct findings through the skin, muscles and bone. Chronic pulmonary disease gives rise to neuritis, muscular pains and arthritis, but generally in a subacute manner. Fig. 1 is a röntgengram of a man who complained of severe pain and discomfort in the ankles, knees, elbows, and wrists. Compared with Fig. 2, a normal chest, it is not a difficult thing to note the wide variance. Fig. 2 shows the typical "moth-eaten" and mottled appearance of tuberculous species: Tubercle bacilli were found in the sputum. He had been sent to me for "Acute-rheumatic-baths," and not for a diagnosis which was too late,—he died.

The gastrointestinal tract affords several places in which pathogenic bacteria can culture and emit their toxins. The gall-bladder, appendix and colon are the principle ones. The diagnosis of infection, acute or chronic of any one of the three is a volume in itself and can only be mentioned. Here intestinal

stasis plays an important role. Abscesses of the fallopian tubes, in and about the rectum and kidneys are frequently foci of infection. Inflammation of the sciatic nerve, so-called "sciatic rheumatism" is not sometimes but always, secondary to a focus of infection, near or remotely situated. If this focus is not found there are the two old faithful alibis, trauma or the weather, for the lazy doctor to blame it upon. These excuses seem to never wear out. Let us dis-



Fig. 1.—Mr. P. V. Heart normal. Lungs, bilateral tuberculosis. Note that both upper lobes are "moth eaten." This condition is also called "mottling." This case died two weeks later. Autopsy findings confirmed clinical findings and diagnosis.

card them. Let us begin our reasoning with a premise like this; there is no such a disease as rheumatism and strike the term from medical nomenclature. If we do so our conclusions will not be far wrong. If one careful search from scalp to toes does not reveal the trouble let us search again and again. If we cannot recognize it let us be fair and say we do not know.

Routine and methodical examinations are all very good but we must keep

our eyes open. We must not permit ourselves to be misguided as I was in the following case:

E. A., salesman, aged 26, complained June 20, 1915, of "articular rheumatism." January 5, of the same year, he had experienced a severe attack of polyarthritis and spent six weeks in bed. He stated that "tonsillitis always preceded the pain in the shoulder, knees and feet." Physical examination revealed submerged and diseased tonsils, temperature 99, pulse 110; urine—a trace of

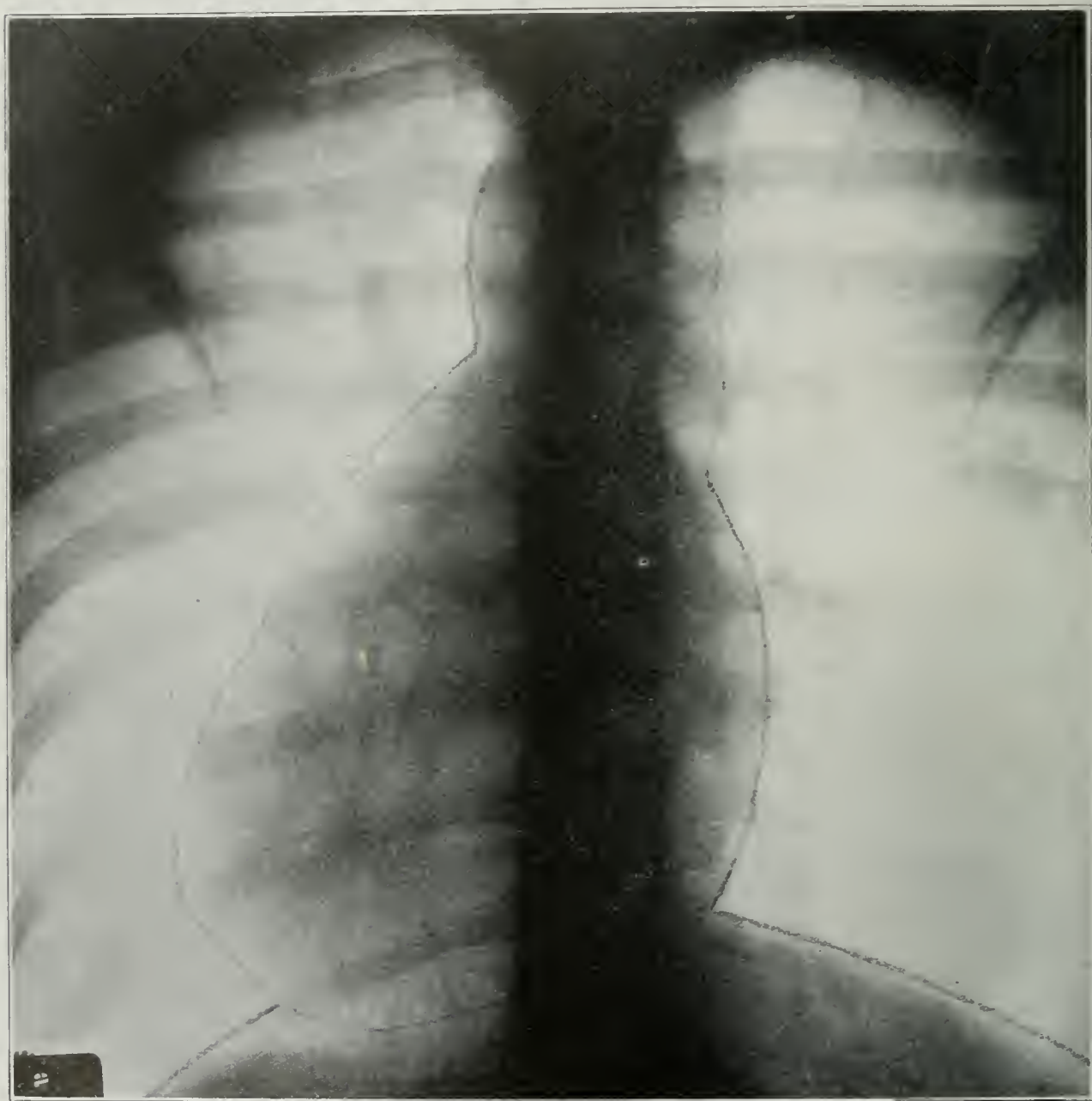


Fig. 2.—Mrs. S. Lungs and heart normal.

albumin and one or two granular casts. I could not make up my mind that the tonsils were the only cause of the trouble, so I went over him three times from "hair to toe nails" before I discovered an abscessed root of the left upper incisor, which he promptly stated "was one of long standing." Streptococci were found in the exudate. His dentist would not extract the tooth, but said he could save the root by treating it. This pleased the patient greatly. His tonsils were removed. Two months later an acute attack of arthritis occurred in his right knee; the "tooth" was still discharging. I took him to another dentist

and had that tooth removed; two days later the knee was well and has remained so ever since (April 26, 1916).

Focal infection in itself may be but a minor thing, but the complications are severe, oftentimes resulting in permanent injury and even in death. Hence the "rheumatic," syphilitic, and nephritic heart; the nephritis, cholecystitis, etc., etc. It is not reasonable that they were primarily affected. We now believe gastric ulcer to be infective in origin and that the avenue of infection is the blood stream; where did it come from, tonsil, tooth, lung or prostate? It is for the physician to find out.

A chronic running ear is but a little thing—perhaps you have seen them of long standing and the patient seemingly suffered little or no ill effect. I saw one once in a woman of 62 years. She stated "my ear has been discharging off and on for nigh onto thirty-five years," and she seemed to have no ill effects from it. Last January I visited a patient, who complained of her "spine hurting." Her people could not get the idea out of their heads that a fall in December was the cause of the complaint. She had been acutely ill but two days. Her physician thought a consultation unnecessary. Physical examination revealed marked tenderness over the right mastoid. T. 100. P. 90. Patient desirous of being undisturbed. She stated her "right ear had been discharging for sir years but had stopped running three days ago and had been paining some since." Spinal puncture and analysis of the fluid showed clearly that the patient was suffering from suppurative meningitis. The meningitis was undoubtedly secondary to suppurative mastoiditis which was secondary to an acute exacerbation of a chronic suppurative otitis media, the focus of infection, streptococci in origin; three days later she died.

There is a focus of infection I have not mentioned—abscesses of the skin and its integument. I have had the good fortune to diagnose and see operated eight perinephritic abscesses. In six of the cases a subacute or nearly healed furuncle or furuncles were found. In all six staphylococci were found in the pus from around the kidney. In one case there was a deep seated ulcer (traumatic) of the right thumb which was "scabbed-over;" staphylococci were found under the scab and in the perinephritic pus. The eighth case was secondary to a stitch abscess complicating an inguinal herniotomy. Colon bacilli were found in the pus of the stitch abscess and in the perinephritic abscess.

In conclusion; then let us make a thorough examination of our patients. Let us at the first opportunity afforded us suspect a focus of infection. If we can, let us find and eliminate the infection. If we cannot find it, let us be honest and say so.

A CASE OF PROGRESSIVE LENTICULAR DEGENERATION WITH AUTOPSY*

BY CHAS. HUNTER, M.D., M.R.C.P., AND S. J. PEIRCE, B.A., M.D.,
WINNIPEG, CANADA.

L. B., a girl of 18, sent by Dr. Swallow, of Russell, Manitoba, admitted to Winnipeg General Hospital on December 22, 1914, and died January 14, 1915, of erysipelas.

She was the third of a family of ten: four sisters alive and well, two dead, one at five and the other when two days old; one brother died at one year, one is healthy and the other, a man of twenty-four, began to shake and walk stiffly in the last six months. (This is confirmed by Dr. Swallow.) Patient said herself that "her brother was going the same way."

Father died of an accident at fifty-one; mother at thirty-eight, after a few days' illness; mother had always been "rather weak." Apart from the brother mentioned, no history of nerve trouble in the family.

Patient was always strong and healthy, and attended school until fifteen years of age when she left in the usual grade and began to help at home, both in the house and at outside farm work.

She menstruated only once, and then three years ago for one day only. Two years ago she noticed that she could not thread a needle or peel potatoes, owing to shaking of the fingers and hands; this shaking was present mainly when she tried to do such fine work and did not trouble her so much when she was busy at outdoor farm work. Over a year ago, she began to get stiff—noticed she had difficulty in getting into bed and in turning in bed, owing to the stiffness. She remarked, too, that her mouth began to keep open in spite of herself and that at times the saliva would dribble, while her friends noted that she smiled a great deal and that for over a year, her speech was indistinct—they often told her to speak clearly. For some months she had needed help in dressing and undressing; the stiffness was worse in the morning; she could walk around with ease, but owing to the shaking which had increased and the stiffness, she could do very little work in the house—only sweeping the floor or scrubbing, and that with increasing difficulty. Recently, in stepping backward, she found she had difficulty in checking the backward movement and sometimes continued to move backwards till held up by the wall. A year ago, she had an attack of measles, and was in bed then for a month, owing to "weakness." The shaking was less on getting up. No dyspeptic symptoms at any time and never any difficulty in swallowing.

CONDITION ON ADMISSION.

She was a well built and very well nourished young woman, with full bust development and pubic and axillary hairs. Lungs normal. Heart normal but rather rapid (80-92); no anemia; leucocyte count 9,600 (polymorphonuclears 68%, lymphocytes 26%, large mononuclears 6%); urine normal; gastrointestinal track negative; no evidence of cirrhosis of the liver; test breakfast gave free

*From the Winnipeg General Hospital.



Fig. 1.



Fig. 2.

hydrochloric acid 45 and total acidity of 70; Wassermann on blood doubtful (2.0.0.0.0.) Temperature on three occasions before erysipelas developed, reached a little over 99 degrees.

Her facial expression was striking and is well shown in repose in the accompanying photograph (Fig. 1). The mouth was constantly open, upper incisors and canines visible with a little of the gum, upper third of front lower teeth also visible with the large tongue very slightly protruding and occasionally slight dribbling. She smiled very frequently and often without any cause; then the mouth opened a little wider, the upper teeth and gums much more exposed and lower teeth less so. The smile lasted long. Apart, however, from this change around the mouth, the facial expression was absolutely fixed and reminded one constantly of the expression in paralysis agitans. The upper eyelids drooped as shown; there was, however, no increase of the drooping as the day went on, as in myasthenia gravis.

CRANIAL NERVES.

Vision and optic discs normal. Pupils equal, medium, reacting to light and accommodation. Ocular movements normal. No nystagmus in any direction.

Corneal reflex brisk on both sides; no sensory impairment over the face. The patient could close the mouth, but did so slowly and relaxed rapidly. She could not whistle.

Hearing was normal. Palate moved well and symmetrically; palate reflex present. Swallowing unimpaired and no difficulty at any time admitted.

The sternomastoids were always hard and did not relax even when she was lying still; she could move the head only slowly from side to side, often with tremor of the head and arms—there was marked stiffness of the muscles of the back of the neck.

The large tongue was protruded slowly but only a little way and the movement was accompanied by slight tremor of the tongue and lips.

Speech was indistinct and monotonous; some days it was moderately distinct, but on other days one had frequently to ask her to repeat what she said.

MOTOR SYSTEM.

The muscles were everywhere well developed and possibly a little firmer than usual to palpation. She walked with ease and with only a trace of rigidity in her gait; no ataxia; Romberg negative. When standing still, however, and pushed gently backwards, she could not recover herself but would continue to move backwards, sometimes till held up by a pillar or wall some distance away. With practice in the hospital, this symptom improved considerably. In startling contrast to her freedom in walking, was her extraordinary helplessness in bed—she turned round with the greatest difficulty and often stopped when half-way from supine to prone position in a helpless, appealing way. She assisted herself in turning with her hands on the head of the bed. Similarly, when getting into bed and rising up from bed, her movements were slow, helpless and rigid in the extreme. The movements of the spine, when she was up, were free: she could bend forward and laterally with ease and this combination of freedom of movement when up, with apparent rigidity on turning in bed forcibly struck one, suggesting hysteria.

When exhibiting the patient at the Hospital Clinical Society as a case of progressive lenticular degeneration, I was disconcerted, on asking her to lie down on the floor and to get up, to find she could do so with comparative ease, rising, however, from the hard floor like a pseudohypertrophic patient. I found later she was aware that she had little difficulty in turning on, or getting up from, a hard floor as opposed to a soft bed.

She needed to be helped to dress and undress while in the hospital; she could put on and take off her clothes, but only extremely slowly, with marked rigidity and some tremor.

A certain amount of rigidity seemed everywhere noticeable in the muscles of the extremities—e.g., in the lower limbs, in flexing and extending or rotating the thigh and in the upper extremities, in flexion and extension of the elbow and in pronation and supination. All movements could be actively and passively carried out—there was no contracture, though she tended to carry the hands slightly flexed at the wrist-joints and extension of the hands at the wrist-joint was definitely weak. The rigidity was slightly more marked in the left upper and lower extremities than in the right.

The muscles of the abdominal wall were quite relaxed, when patient was lying in bed and the abdomen could be palpated with comparative ease.

Generally, no tremor was noticeable; on excitement or on muscular effort, a marked rhythmical tremor developed, mainly in the left upper extremity, though when marked, involving the head in flexion and extension, the left ankle and toes in flexion and extension, and slightly the right upper and lower extremities in a similar fashion to the left. The tremor particularly developed when she tried to put on or to take off her stockings and was well marked in the finger-nose test. Protrusion of the tongue developed a slight tremor of the tongue and lips. In the left upper extremity, where the tremor was most marked, it took the form of alternating flexion and extension at the elbow, flexion and extension at the wrist and alternating pronation and supination to a slight degree; its range varied with the muscular effort and excitement and sometimes reached 2"—3". Patient could control the tremor sometimes when ordered to do so; at other times, the command aggravated the condition.

SENSORY SYSTEM.

No alteration in any form detected and no subjective sensation.

REFLEXES.

The abdominal reflexes on the left side were never elicited, though frequently examined; on the right side, the upper abdominal reflex was elicited repeatedly but never the lower. Knee-jerks and achilles-jerks brisk and equal; no ankle-clonus, though a pseudoclonus was repeatedly obtained. A definite extensor response was elicited repeatedly from the sole, particularly on the left side, but more frequently the response was of the usual flexor type. Sphincters normal.

During her three weeks' stay in the hospital, patient answered questions intelligently and took quite an interest in the other patients, who remarked on her frequent smile.

She was quite cheerful but remarked repeatedly that the doctors had not

helped her so far. The nurses thought she was rather simple and childlike. On January 11 she developed facial erysipelas, which spread rapidly over the head, her temperature rose rapidly to 105 degrees and before death to over 107 degrees; sensorium clear but progressive cardiac weakness—death on January 14, 1915.

Autopsy was performed January 15, 1915, by Dr. Peirce.

SYNOPSIS OF POST MORTEM FINDINGS.

External Examination.—Body of young adult female, well developed and well nourished. Upper portion of face edematous. Bleb-formation on lower portion of forehead, left ala of nose and lobe of right ear.

Internal Examination.—The only organs that show changes other than are incidental to septicemia are the liver, spleen, and brain.

Liver.—Markedly cirrhotic. This is best seen on the lower surface which

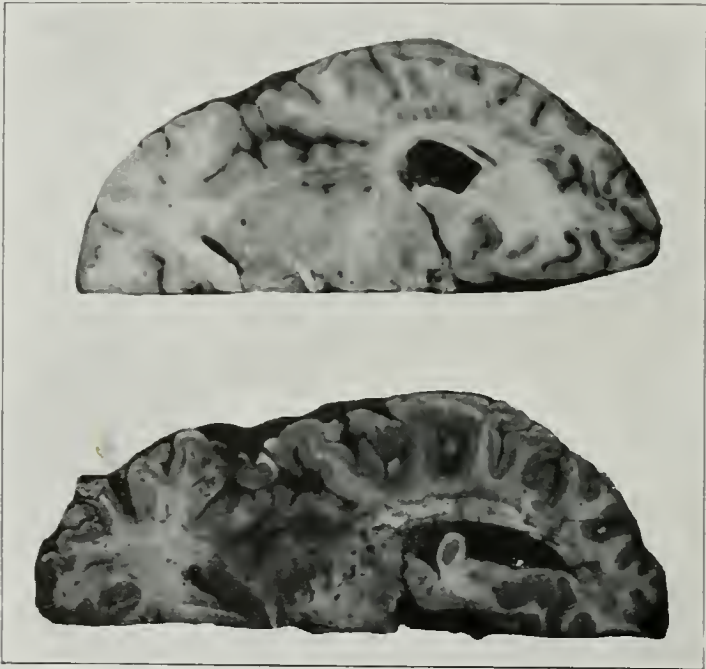


Fig. 3.

presents a multitude of small lobules 2-10 mm. in diameter, separated from one another by bands of connective tissue. Organ is somewhat smaller than normal and of firm consistence. Weight—1,402 grams. (Fig. 2.)

Spleen.—Uniformly enlarged, soft, but pulp is not defluent. Weight 355 grams.

Brain.—Cerebrospinal fluid somewhat excessive in amount. Vertex of right hemisphere shows a thickening of the arachnoid, which appears as a milky area about one inch in diameter. Extreme anterior poles of both hemispheres show single, symmetrical, cord-like adhesions of arachnoid to dura.

Weight after hardening in ten per cent formalin:

Left hemisphere	639 grams.
Right hemisphere	650 "
Cerebellum and pons.....	167 "
Total	1,456 "

Horizontal section along a plane immediately below the extremities of the corpus callosum (Pierre Marie's coupe d'élection) gives the appearance shown in Fig. 3. The figure below is that of the left hemisphere of the brain. The figure above is for comparison, and is that of the left hemisphere of the brain of a boy who had died of tuberculous meningitis. The only noteworthy feature of the section is a uniform diminution in size of the lenticular nucleus. The reduction in size seems to be confined largely to the putamen. The normal pattern of the cut surface here is blurred by a brownish foggy appearance which extends outwards into, and to some extent obliterates, the external capsule. The nucleus presents no cavitation nor does it appear softened. The cortex appears everywhere of normal thickness. Gross serial sections reveal no other abnormality.

Spinal Cord.—Beyond old adhesions of the meninges no gross change can be made out.

Microscopic Examination.—Section of the lenticular nucleus (Van Gieson and Ehrlich's acid hematoxylin) show a dearth of nerve cells, and an increase in glial elements. The nerve cells that persist show mostly an eccentric nucleus and brown granules in the cytoplasm. The vessels show no perivascular infiltration and no endarteritis. Small mononuclear cells containing brown granules are fairly numerous.

Section of Cord.—(Osmic acid). Show no demonstrable areas of degeneration. Cells of the anterior horns appear normal. Meninges show no infiltration.

Sections of the liver confirm the gross appearance. There is great increase in connective tissue elements. In many places the liver substance is broken up into isolated islands of liver cells by bands of connective tissue. Many liver cells show fatty change.

Remark.—No change was found microscopically in the internal capsule or in the pyramidal track in the cord to account for the occasional extensor response and the constant absence of the abdominal reflexes on the left side.

These physical signs presented a difficulty in regarding the case as one of "Progressive Lenticular Degeneration," as described by Kinnier Wilson in "Brain" in 1912.

LABORATORY METHODS

RADIOGRAPHIC STUDIES OF CEREBRAL VASCULAR LESIONS. A PRELIMINARY NOTE*

BY CHARLES O. DHONAU, CINCINNATI, OHIO.

MUCH has been written concerning the vascular lesions in the cerebral vessels and very many of the usual photographs have been used to illustrate cerebral lesions due to vascular disease, but so far as I have been able to discover no previous attempt has been made to study the vascular system of the brain as a whole by means of injection and x-ray photographs. This is perhaps more to be wondered at because in studies of the vascular system of the heart the method has been of considerable value, and when it is recalled that the brain suffers at least as frequently as the heart from diseases of its vessels it would seem that in this viscus the same methods would be at least of equal interest. It is, perhaps, the fact that the technical difficulties in injecting the brain after it has been removed from the skull are greater than in most other organs. There are so many collateral communications which must be broken in removing the organ, that the injecting fluid or mass leaks out in innumerable places. The waste is therefore great and in dealing with injection masses which are satisfactory for x-ray work artifacts are very easily produced and these are often misleading. Therefore, the method which was worked out by me involved leaving the brain *in situ*. By this method the brain could be thoroughly washed out, then injected readily, and finally removed with little danger of leakage provided a proper mass was used and other necessary precautions were observed.

The matter of the injection mass gave a certain amount of trouble. A successful mass is one which must form a fairly stable suspension and which will, after fixation of the brain, be impenetrable to the x-rays. Jamin and Merkel¹ in injecting the coronary arteries used red lead wax and turpentine as one mass, and red lead iodipin and wax or gelatine as another. A third mass was composed of a heavy proportion of red lead in a 10-15 per cent solution of gelatine. Experimentation with these masses showed in the case of the red lead, wax and turpentine, that constant agitation of the mass was necessary during the entire injection. The experiments with gelatine were as a whole unsatisfactory on account of the effects of temperature on the viscosity of the mass. While in the case of single removable organs these masses were utilized in a satisfactory manner, the injection of a circulation as extensive as that of the brain from a point quite distant, as in the case of my method, required an injection mass which was stable and which was not affected by variable temperatures.

Hauch,² injecting the renal arteries, used 500 grams of red lead, 500 grams of paraffine oil and 250 grams of turpentine oil. Experimentation with this mass showed that it had to be agitated during the injection to prevent an uneven con-

*From the Pathologic Institute of The Cincinnati General Hospital.

centration of the lead. The plates made from the organs in which this mass had been injected were fairly satisfactory. On account of the inherent weaknesses of both the Hauch and Jamin Merkel masses for injection, I experimented with the injection mass suggested to me by Dr. Herbert A. Brown, Curator of the Museum of the Pathologic Institute of the Cincinnati General Hospital. This was a suspension of litharge in glycerine. This proved to be a perfectly homogenous mass and in the proportion of 225 grams of litharge to 500 c.c. of glycerine penetrated into the principal medullary branches of the cerebrals. This mass threw an even shadow and the plates showed a clear delineation of the arteries from the Circle of Willis to the larger branches of the terminal cerebrals.

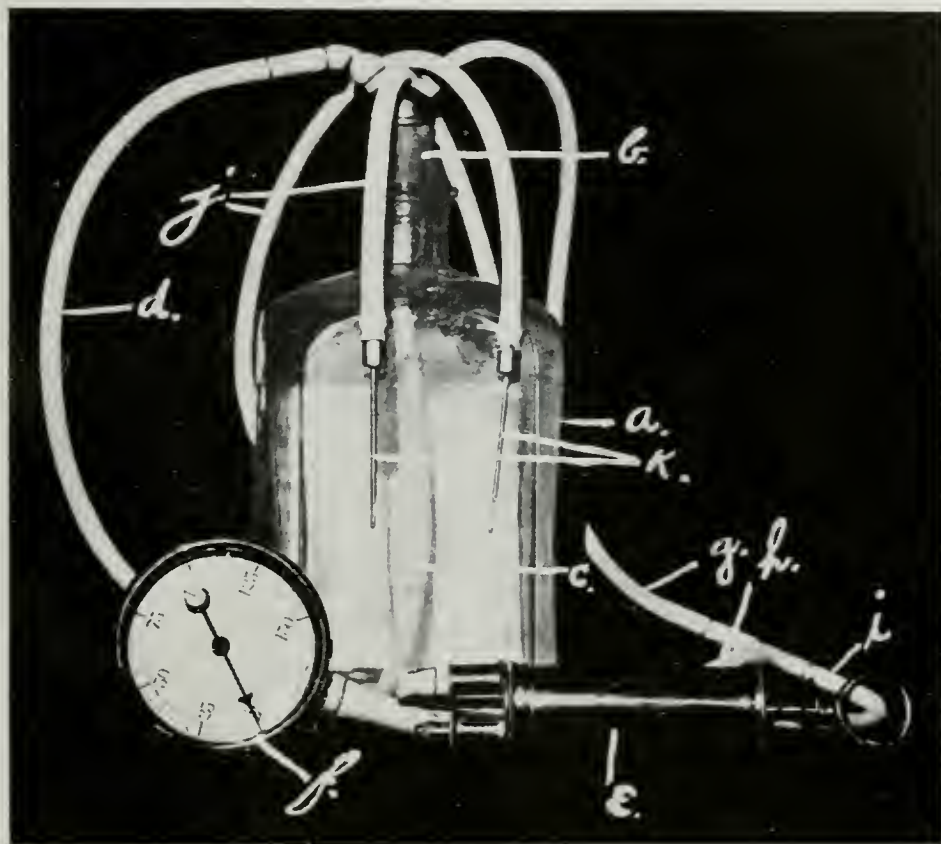


Fig. 1.—The injection apparatus. (a) a 64-ounce squat injecting bottle; (b) a rubber stopple into which was fitted a metal gooseneck; (c) a rubber tube extending from the gooseneck to the floor of the bottle; (d) a rubber tube extending from the gooseneck to a Y connection; (e) a hard rubber aspirating and injecting pump; (f) a pressure gauge; (g) a rubber tube from the gooseneck to a Y connection on the air side of the apparatus; (h) a rubber tube from the pressure gauge to the Y connection on the air side; (i) a rubber tube from the air pump to the Y connection on the air side; (j) 2 rubber tubes from the Y connection on the injection side to the canulae; (k) 2 metal canulae.

The technic of the injection was as follows: The right and left common carotid arteries and the right and left internal jugular veins were brought to the surface at the upper margin of the sternum. Canulae were introduced into both carotids. Incisions were made into both internal jugulars for drainage. The external carotids were ligated. An injection of 2,000 c.c. of one per cent solution of borax was made simultaneously into both carotids. The injection of the borax solution cleared the blood from the arteries and sinuses with the exception of such blood as was in the form of thrombi. The washing was followed by an injection of the litharge-glycerine mass. This last injection was started at low pressure and was continued until the maximum resistance was noted. Maximum resistance in this form of injection equaled a pressure of 18 pounds to the square inch. The amount of the mass injected before the point of maximum resistance was

reached averaged 350 c.c. After the injection was stopped, the canule were removed from the carotids and the arteries were ligated. The head was placed in a position of retraction during the entire injection. Immediate cranial evisceration was then done by the usual method practiced at postmortems. On incising the scalp the injection mass exuded from the arteries. On retracting the scalp the temporal arteries were found fully injected. On removing the calvarium (which was done without injury to the brain substance) the meningeal arteries were found fully injected. On removing the dura the terminal branches of the cerebrals were found fully injected. To prevent the escape of the injection mass the internal carotids and basilar artery were ligated before division. The viscus was then removed from the skull and was placed in a specimen jar containing a 10 per cent solution of formaldehyde and sufficient cotton to give

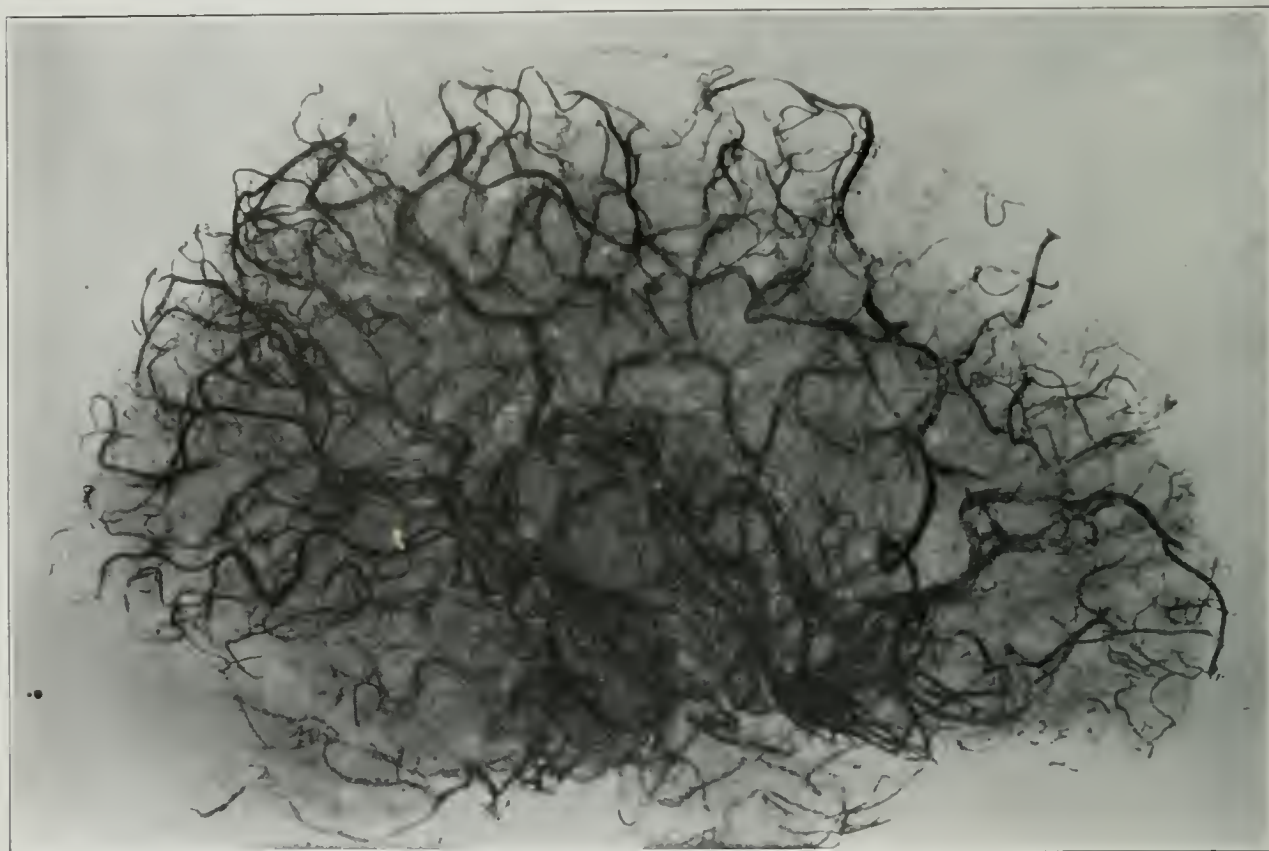


Fig. 2.—This is a normal specimen in which there is no vascular disease. Note the regularity of the arteries as compared to those in Fig. 3.

support to the brain. After an immersion of 48 hours it was removed from the specimen jar and the exposure to the x-rays was made. Two exposures were made. The first was of the entire brain with the base toward the plate. The second was of both hemispheres with the mesial surface of each toward the plate. With both hemispheres on the same plate the lateral view was found to be of much value on account of presenting both a normal and an abnormal specimen for differential study.

To illustrate the results of this method, photographs of two plates are presented (Figs 2 and 3).

There was a clinical diagnosis of cerebral hemorrhage. The autopsy diagnosis made without sectioning the brain was arteriosclerosis; diffuse subarachnoid hemorrhage from the right and left posterior cerebellar arteries; throm-

basis of the right and left posterior cerebellar arteries. After the exposure to the x-ray the brain was sectioned, showing, a mass of clotted blood which entirely filled the left lateral ventricle; an area of white softening in the left hemisphere corresponding to that portion of the medulla which is supplied by terminal branches of the ascending frontal artery. There were no pathologic changes in the right cerebrum. The x-ray diagnosis was arteriosclerosis, the vessels showing marked tortuosity and irregularity; rupture of the left middle

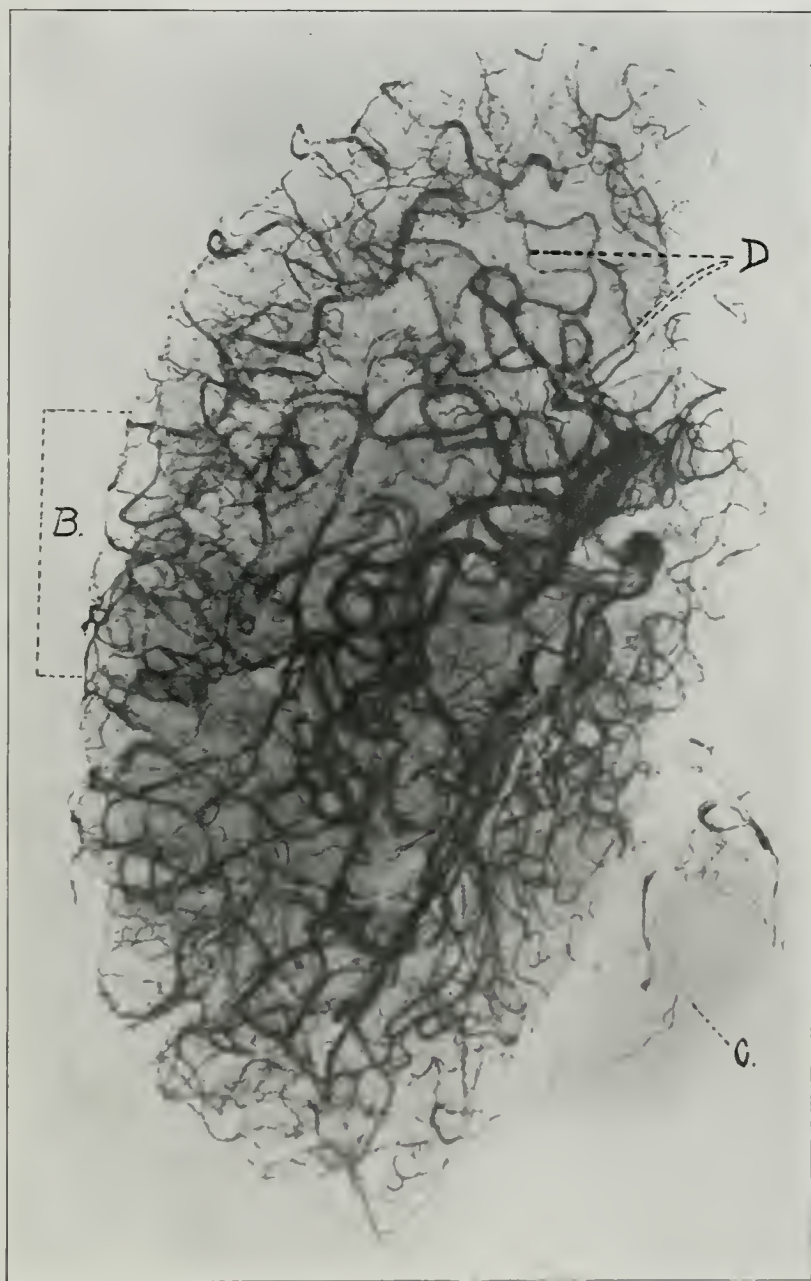


Fig. 3-A.—Case No. A-2997.

cerebral artery at a point in its main trunk, as shown by an apparent projection into the intima of this artery of a part of the clot, when seen stereoscopically; thrombosis of the posterior cerebellar arteries, as seen by the absence of the injection mass in these vessels; thrombosis of the terminal branches of the ascending frontal artery on the left side, as seen by the absence of the injection mass in these vessels; possible multiple miliary aneurismal formations in the right cerebrum. To demonstrate these findings, I have marked the print as follows:

(A) Area of thrombosis of the terminal branches of the ascending frontal branch of the left middle cerebral; (B) Corresponding normal area in the right side; (C) Thrombosis of the right and left posterior cerebellar arteries; (D) Possible miliary aneurismal formations.



Fig. 3-B.

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STUPID WORDS AND STATEMENTS COMMONLY APPEARING UPON LABORATORY REPORTS

BY B. G. R. WILLIAMS, M.D., AND E. M. WILLIAMS, B.S., PARIS, ILL.

A SLIP of the tongue is often overlooked, but a slip of the pen is regarded as inexcusable. A laboratory report, standing as it does as a permanent record of precise data and not to be altered save by amendment provided by re-examination, should be strictly accurate.

Yet to study or even glance many reports, the reader is often led to doubt if the examination was really worth while. The family physician desires a usable record of diagnostic worth. Hoping for this he has sacrificed time and patience in collecting and preparing the specimen. The sick man has provided the "good iron dollars." The laboratory man, intensely interested in the problem, has made the examination and has determined (to his satisfaction) what, if any, light it throws upon the diagnosis. Yet, the permanent record raises innumerable questions, and finally leads those who have not seen the work carried out, to doubt the value of the examination and to discredit laboratory work in general. No matter how skilled the examination, it frequently happens that the written report is valueless.

THE EQUIVOCAL "NEGATIVE" AND SOME OTHER WORDS.

A test may be positive or negative, but these terms, and especially the latter, are much misused. The odor of a urine is rarely negative, the conclusions on a sputum examination (embracing as it should many tests) should never be stated by a terse "negative" and a differential blood count is never negative. Negative is an adjective and implies the absence of certain qualities. It may be used in complement-fixation work, for example, to show that specific antibodies or complement-binding qualities are absent from the patient's serum. But it seems to us that its use to express the fact that a color is normal, that the worker is unable to find tubercle bacilli in a sputum (pus, blood and other important findings may be present) and its promiscuous use on clinical reports, is not justified.

The word "precipitate" (noun) is often used incorrectly by men who should set a standard for accurate, scientific expressions. Truly speaking, coagulated albumin is not a precipitate but is a coagulum, the solid or semisolid state of a sol. The term "precipitate" should be restricted to the solid state of a crystalloid as thrown from true solution.

It is our observation that "mucus" and "mucous" are quite generally confused or spelled incorrectly.

"Blood," "hemoglobin," "hematin" and "erythrocytes" should be more carefully differentiated in laboratory reports. This criticism is not unjust, for it may have some bearing upon the diagnostic use of the information.

The term "found" is not strictly incorrect, but in chemical work "detected" is perhaps preferable whereas in microscopy the term "identified" seems to us

to be more desirable. It is likewise merely a question of preference when we advise the use of the words "sensitive test" rather than "delicate test."

"Trace" is not definite enough, for what one worker may consider a trace, the next will report as absent. The latter may report as a trace that which the former would regard as a small amount. "Trace" should always be qualified as "questionable trace," "appreciable trace," "marked trace," and "more than a trace." This suggestion is volunteered because the average practitioner is inclined to disregard the word "trace" and dismiss it as significant of no clinical meaning.

"Present" may be permissible in some portions of the report but is not definite enough as a rule. It seems to us that it is not sufficient to report casts as "present," but these casts should be classified and an estimate of the number per field should be made. The reason is obvious: a few hyaline casts may have but little meaning, but where a dozen or more granular casts are present in every portion of the drop the case is different. Moreover, it may be possible in a series of examinations to approximate a prognosis. Elsewhere we have shown that by such series of examinations, vascular nephritis may be diagnosticated by the study of the urine alone.

REPORTS OF URINALYSIS.

Amount.—While every laboratory worker would prefer to know the actual amount of urine being passed each twenty-four hours, it is not always possible to secure these figures. The blank which provides for this, should not be filled in with the word "negative," but should be marked "Amount not stated." If it is probable that the amount is normal, but there is uncertainty because figures are not available, the sentence "Probably normal, according to patient's statement" or "Probably normal, as suggested by other properties" will leave no question in the mind of the reader.

Color, odor.—We doubt if the color of a urine should ever be stated as "normal," but concede at the same time that it is very difficult to find a satisfactory word to express the color. It has been very difficult for laboratory men to agree upon a chart or scheme of normal and pathological urinary tints. Moreover a color which might be normal for a twenty-four hour specimen of 48 fluid ounces, would by no means be normal for one of 10 fluid ounces or 100 fluid ounces. The color of the sample may be unimportant or absent but in either case the word "negative" should be avoided since it has become ambiguous through usage. The same principles apply to the designation of the odor of the urine. We fear that the man doing much urinary work is becoming a bit careless in the use of words which may mean much to him, but mean nothing to the family physician or scientific readers in general.

Solids.—Total solids should always be qualified by reporting as "per twenty-four hours" or "per liter."

Reaction.—It seems to us that where litmus is not used as the indicator, that the name of this indicator should be stated with the results.

Albumin.—The more progressive laboratories are now reporting albumin as serum albumin in order to discriminate between it and other albuminous bodies. We routinely report it in filtered sample, but further qualify if it seems

to be explained by pus, blood, and so on. We eliminate the question of bacteria by refusing to examine decomposed samples, by pointing out this source of error in the report or by shaking with calcined magnesia, filtering until clarified and controlling the tests carefully. When we fail to detect serum albumin in a sample of urine, we do not use the term "negative," but state that it is absent by such and such a test or by the routine clinical tests. It seems to us that the term "albumen" should be dropped.

Other Chemical Tests.—"None" or "O" is usually employed by us to show that Bence-Jones body, bilirubin, glucose and so on are absent.

Inasmuch as the purpose of the laboratory report blank is to serve as a reminder as well as a convenient means for classifying the findings, it seems that it is well to provide space for all tests of known value. In every examination, however, it is not necessary for the worker to set up diazo, pentose and urochromogen tests. In such case it is not proper to fill in these blanks with the word "negative" (and this is being done by some men), but it is much better to draw lines through these words, showing that the respective tests were not made. The laboratory man is not called upon to fill every blank in the report sheet, and is unfair to himself and his clientele if he attempts to do so.

Quantitative Work.—In the same connection comes the question of how much quantitative work the laboratory man carries out in the routine urinalysis. Most men estimate the solids, if provided with twenty-four hour figures, and some calculate the urea. Well and good, but a large number likewise fill out all the blanks in the quantitative section—not with figures, of course, but with the words "present" or "absent." What stuff is this? Many of our laboratory men (and we judge by their reports) find urates, chlorides, sulphates and phosphates in samples of urine submitted to them for diagnostic examination. Perhaps these same men oftentimes wonder why practitioners lay so little emphasis upon the value of their reports. We will not be surprised one of these days to find water reported as present in some urinary sample.

Microscopy.—The laboratory man is at his best in reporting microscopic findings. The practitioner has learned this, and often instructs him to make only the microscopic study.

However, there is one point which must be straightened out eventually and this perhaps will be realized only by a general agreement. This is in regard to the reporting of pus cells or the various types of white blood cells in the urinary sediment. Show us the report that states that pus cells were absent, and we can usually prove that the worker was either careless and not thorough in his examination or that he possessed intelligence enough to ignore the few leucocytes he saw that the family physician might not be confused thereby. Here are some rules in this connection which are not to be taken as final, but are designed to open up the question anent the reporting of pus cells:

1. The presence of many of these cells (at least two in every high power field, unless found by low or high power in clumps of five or more) should be reported not only as such, but the point should be emphasized that "this urine contains true pus—case of pyuria."

2. If possible, distinguish between small renal cells and the various types of white blood cells, and so state upon the report.

3. Attempt to determine the cause and origin of the pus not only for the diagnostic value of such a procedure, but to actually determine in a given urine where there are a few or more than a few white cells, that these are truly pathological and that pyuria should be reported.

4. Never report cancer cells in a urine sediment. Malignancy is determined, not by cell types but by cell relations. Cells desquamated from a benign papilloma may present a vegetative appearance but may not have broken through the basement membrane at any point.

SPUTUM REPORTS.

There is nothing more disgusting to us than some sputum reports. Not a few of these come from laboratories of men who really know better, but who perhaps become careless. We have witnessed reports with the terse, "negative" stamped, written or typed followed merely by the signature of the examiner. It is perhaps not for us to say whether the worker shall or not report upon elastic tissue, cells, albumin and so on; it may be his privilege to regard a complete sputum examination as a search for the bacillus of Koch. But we do object to the meaningless "negative" passing as the last word from the medical laboratory—the sick man deserves a better fate. We make it a rule when reporting tubercle bacilli as absent, to give the physician some idea as to what kind of a search we carried out (and there are various methods carried out). Thus, for example, we state, "absent in three smears examined closely," or other words which will mean something to him.

But we have contended and still contend that a sputum examination means something more than to peep through a lens for red rods. The time has come when a sputum examination, done properly, is much more tedious (and fascinating) than a urinalysis, and when properly completed and reported, the fee should be twice or thrice that charged for the urinalysis.

OTHER REPORTS.

In general the same rules as given above apply to blood, gastric, pus and other reports, and it is not necessary to consider these in detail. If medical diagnosis reaches perfection anywhere, it is in the medical laboratory. Here are found the men who dare not guess, but who must work out problems with an accuracy demanded from no other class of specialists. Their records are permanent. These reports may travel across the continent and reach the hands of the greatest clinicians of the world. The future sentence upon the medical laboratory will be passed by the men who are reading these reports, and by these we will be judged.

This plea is to the laboratory man. Prepare well your exhibit for the eyes of the jurors.

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Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
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EDITORIALS

The Diagnosis and Treatment of Paresis

THREE papers lately published pertain to the very interesting subject of the serological diagnosis of general paresis and its treatment by the newer topical methods.

Lowery¹ publishes the results of four years' experience at Danvers with the serum and spinal fluid Wassermann. In 1,714 admissions since May, 1912, serum Wassermann has been done on 1,600 and spinal fluid Wassermann on 276. The cases were unselected. Two human heart, cholesterolin fortified, and one luetic fetal liver extract were used as antigens in each test. Of the sera 16 per cent were positive, 3.87 per cent were doubtful and 80.13 per cent were negative. Of cases not showing a positive serum reaction (i.e., of 5 doubtful and 12 negative sera) 17 gave a positive spinal fluid Wassermann. In this series therefore one per cent of positive diagnosis was gained by performance of lumbar puncture.

It is of interest that 7.6 per cent of cases not clinically paresis gave positive serological results. Of the cases clinically paretic 87.5 per cent were positive, 10.3 negative and 2.2 doubtful. Other psychoses present some interesting findings: 10 per cent of imbeciles were syphilitic and in a group diagnosed

"pre-senile delusional insanity," corresponding perhaps to the familiar "involuntary depression" there were 16 per cent of positive findings. It is also worth noting that in less than half of the positive cases was a positive history obtainable. However, mental incapacity would explain this to a great extent.

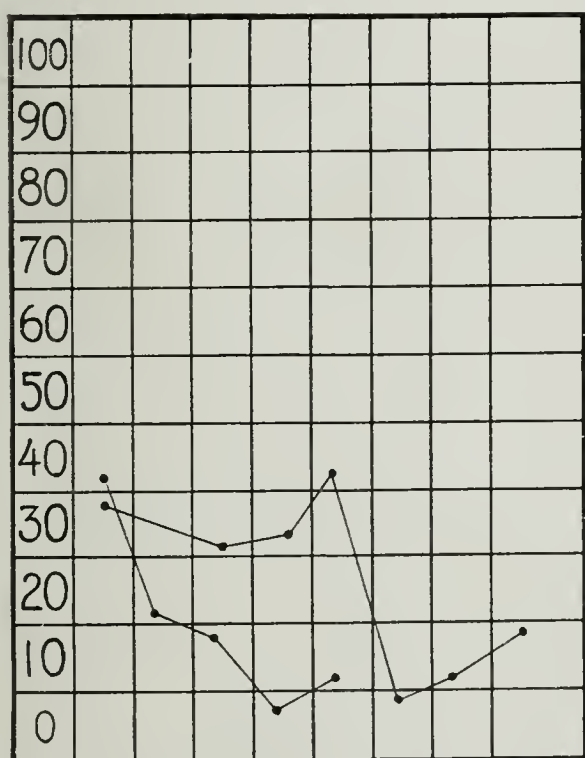
Evans and Thorne² present conclusions of the value of salvarsanized serum in the treatment of 15 cases of paresis; one of which was treated both intraspinally and intracranially. The number of intraspinal injections average about six for each patient. They summarized their results; three patients showed mental and physical improvement, but of these only one has remained well; one has withdrawn from observation and one died of convulsions less than ten months after the conclusion of the treatment; five showed physical improvement only and these two died about a year after discontinuance; three showed no improvement at all. They believe that improvement is no greater than with older methods and point out the fact that mere lumbar puncture will bring down the cell count. However, some objections could be raised to their work and conclusions. They have used the Swift-Ellis technic exclusively. By this method an average of 0.16 mg. of Salvarsan per c.c. of serum is obtained, though variations are enormous and range from 0.004 to 0.05. In Ogilvie's technic from 0.3 to 0.5 mg. are added directly to the serum *in vitro* so that the dose becomes definite and this improvement has been recognized by Swift in his last publication.³ Besides, Evans and Thorne did not resume treatment when relapses appeared. In summarizing they say: "If the treatments are given at shorter intervals one is merely withdrawing part of the serum last injected as it takes nearly two weeks for the serum to be absorbed from the sub-arachnoid space." Though moderately familiar with the literature of intraspinal medication the present writer has never seen any data on the time of absorption and no authority is quoted for the statement referred to. Their conclusions that the intracranial route is unnecessary is based on one case only and is therefore negligible.

Wardner⁴ reviews this method from safer ground, advocating it on the basis of fourteen cases treated by the intracranial method continuing a report on six cases of a year ago. Whenever possible relapses have been met with renewal of treatment and the treatments have totalled 82. His results are quoted verbatim: "To sum up of the fourteen cases five improved sufficiently to go back to their work and, to date, have remained well for from seven to eleven months. At the end of eleven months one of these cases had a bad relapse. He was immediately brought back to the hospital and he responded immediately to additional application of the serum. At present he is well mentally and physically and has parole of the grounds. Three other well developed cases have improved sufficiently to be put on parole of the grounds and are doing efficient work about the hospital. Three others have shown fairly marked physical and mental improvement, but cannot yet be trusted at large. Two have died." It is worth noting that neither died directly of cerebral syphilis, one had bronchopneumonia and one a cerebral cyst certified postmortem and apparently of earlier origin than the treatments.

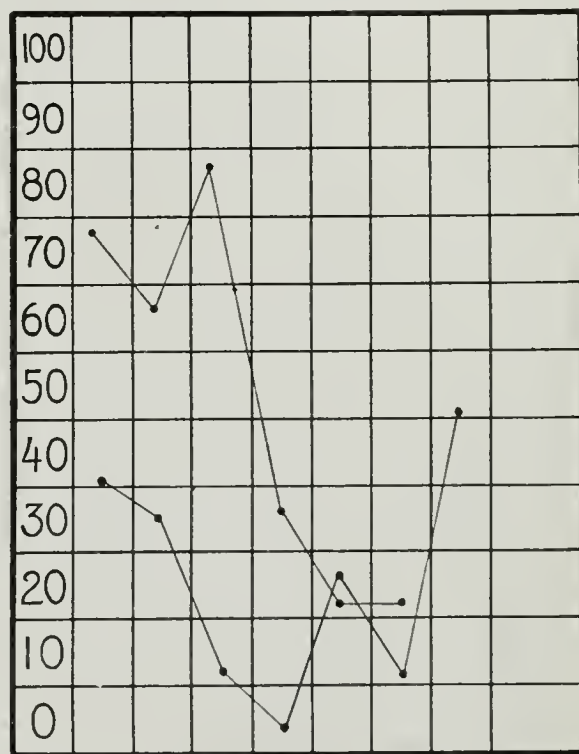
Such poor results as are reported by Evans and Thorne are fortunately not common. It is gratifying, however, that they have recognized the practical importance of the experimental work of Cushing, Weed and Wegefardth.⁵ In a previous note⁶ the present writer called attention to the fact that in the contro-

versy over the value of the various topical methods of treating cerebrospinal syphilis few seem to have realized the point of this research which demonstrated that, with pressures only very slightly and surely not dangerously above normal, the cerebral subarachnoid and perivascular and perineuronal spaces could be injected from the spinal canal. However, to reject the intracranial route on the basis of this and a single personal experience is not justified in the face of the excellent results reported especially by Wardner.

The value of laboratory findings comes in question again. Evans and Thorne contend that the fall in the cell count only parallels that produced by simple lumbar puncture and present "curves" comparing four treated with four untreated cases. Their claim that the curves "correspond well" is scarcely tenable as can readily be shown for their first two comparisons by the simple de-



No. 1



No. 2.

vice of superimposing them as in Figs. 1 and 2. Besides in case 1 while in the chart of the treated case the intervals on the abscissa represent a definite period (14 days), in that of the untreated case this unit is made to represent intervals varying as much as eleven and twenty-one days. If the time factor is immaterial the "curves" represent nothing—if material they are highly inaccurate. Too many and too uncertain factors enter into the cell count to plot it as a simple function of the time. Engineers and mathematicians would smile at a curve such as that of untreated case 4 plotted from four observations! At best these charts are good graphic diagrams but to attempt to compare them as curves is poor medicine and poor mathematics.

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—C. E. Kiely (*Per P. G. W.*).

The Formation of Calculi

PATHOLOGIC calcification occurs most commonly in dead tissue—in areas which have undergone necrosis. It also occurs less frequently in apparently healthy tissue. When the latter happens there is usually associated with it some severe disease of bone. Between calcification as we usually mean it, and concrement formation there is no discoverable fundamental difference. The superficial difference is that concrement formation takes place in a fluid medium, i.e., a more dilute colloidal solution than the one represented by that which forms the basis of areas of necrosis or of normal tissue. In ossification (i.e., normal calcification) the fundamental process is again the same, the only difference being that after calcification the process of organization proceeds in an orderly fashion to produce normal bone. Ossification occurs also under pathologic conditions, and produces true bone which however tends to have a more or less disorderly arrangement. Abnormal ossification occurs in such diseases as rickets and congenital syphilis.

In all these cases the matrix (whether fluid or solid) in which calcification is to take place, is saturated with lime salts, held in solution by virtue of the colloidal condition of the matrix, and so held because of the fact that these salts, chiefly carbonates, and phosphates of calcium (and urea) are more soluble in colloids (albumen) than in water. This may be illustrated by taking a solution of the normal body salts in serum and diluting it with water. The carbonates and phosphates of calcium are precipitated. Variations in alkalinity produce similar results. For instance, if one takes

KCl	0.40
CaCl ₂ + 6 H ₂ O	0.62
MgCl ₂ + 6 H ₂ O	0.37
NaCl	5.90
NaH ₂ PO ₄ + 1 H ₂ O	0.236
NaHCO ₃	3.53
H ₂ O	1000.0

one finds that very soon a precipitation of calcium carbonate and phosphate appears.¹ If, however, serum colloids are added to the solution, no precipitation occurs.

Also Pauli and Samec have shown that, especially in the case of the difficultly soluble electrolytes, the presence of albumen makes a very considerable difference in the salt content of the fluid, as follows:

	In 100 grams H ₂ O.	In 100 grams serum.
Calcium sulphate	0.223	0.226
Calcium phosphate	0.011	0.021
Calcium carbonate	0.004	0.023
Silicic acid	0.023	0.030
Uric acid	0.040	0.057

Bechhold and Ziegler² showed that an electrolyte from serum albumen solution with an albumen content equal to that of defibrinated serum (7.6 per cent) uric acid was much more soluble than in water, while the reverse was true of sodium urate.

	In 100 grams serum albumen.	In H ₂ O.
Uric acid	0.02	0.00645
Monosodium urate	0.055	0.12—0.15

They also remarked that the slightest traces of sodium bicarbonate (in the case of uric acid and of sodium urate) influenced the solubilities.

From the fact that hydrogen and hydroxyl-ions have a remarkable influence upon the water content of albumen, it will be realized that these substances will also have a marked influence upon the amounts of salts held in solution in such fluids as serum and protoplasm.

What happens in ossification or calcification may be that these tissues, living or dead, and saturated to a greater or less degree with serum and salts, are changed sufficiently in reaction, or in water content, to bring about a deposition of the more difficultly soluble salts. Such a process may be facilitated or prevented by certain products of protein metabolism. It has been shown, for example, that the albumoses act almost exclusively upon the calcium carbonate to keep it in solution, while at the same time the phosphate becomes less soluble.³

Were the composition of the fluids of the body the same, undoubtedly we should have but one type of salt precipitation to deal with in studying calcification and concrement formation. As it is, the fluids vary, the common type is that of which we have been speaking, but under certain conditions of metabolism the urates of the body fluids are increased in concentration and then, instead of calcium salts, the deposits are composed of urates, which, like the calcium salts, are more readily soluble in serum than in water. In gout deposits urates are distributed in various parts of the body, and in a condition called "calcium gout," described by Schmidt, deposits of calcium produce an analogous picture. In the fluids of the body, as in the tissues, the type of deposit depends upon the composition of the fluid. In bile, cholesterol and bile salt calculi occur; in the urine, uric acid and urate "stones."

All concrements are formed about a nucleus and are held together by some organic material which acts as a "binding substance." Bacteria, epithelial cells, mucin, a foreign body, coagulated protein, all may act as nuclei about which precipitation occurs, and about it the particles are held together by mucin or fibrin, or perhaps other substances. In the case of urine, it must be remembered that very little colloidal material is present, so little that oftentimes not sufficient exists to bind together precipitates. When this is true urinary sand is formed instead of calculi. Moreover what binding substance is present is apt to be of a reversible nature, and therefore is inefficient. In the presence of inflammation,

however, fibrin, which is irreversible, is produced and this forms an excellent material for binding precipitates. In this connection it is interesting that H. Schade in his experiments with urinary calculus formation, found that fibrin was absolutely necessary.⁴

In the case of gall stones, apparently the only necessary things to assist calculus formation, are fats or fatty acids, for a few drops only of an oil or fat is all that is necessary to produce a precipitate of cholesterin from a solution. The precipitate is first an amorphous mass similar to that which Naunyn believed to be the starting point of calculi. Following this effect radial crystallization occurs about the amorphous calculi. The necessary fats can be produced by splitting of the fatty acid salts and cholates which are present in bile, and this splitting can be accomplished by *B. coli*, *B. typhosus*, *B. pyocyaneus* or *B. proteus*. Staphylococcus does not do this. If it be said that oversaturation of the bile is necessary before precipitation occurs, it may also be said that any reduction of the normal alkalinity of bile produces such an effect, and that growth of various organisms may accomplish such a result. The most important bacterial causes of gall stones are bacteria of the colon-typhoid group which act in reducing alkalinity, splitting the cholates and soaps of the bile, and adding some fibrin to it. Accordingly they satisfy all the needs of the situation in the production of cholesterin stones. But many gall stones contain also bilirubin and calcium in large amounts, a combination which does not occur normally in the bile. In weak alkaline solutions, calcium combines with bilirubin, especially in the presence of certain albumens. In catarrhs of the gall bladder in which the mucus content is increased, the alkalinity is increased and accordingly the conditions are satisfactory for the production of bilirubin—calcium calculi. In the case of calculi of mixed structure, variations first in the direction of alkalinity and then toward acidity give the necessary conditions.

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—P. G. W.

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No. 12.

ORIGINAL ARTICLES

POISONOUS PROTEINS*

(Continued from page 643.)

BY VICTOR C. VAUGHAN, M. D., ANN ARBOR, MICH.

Part II.—Vegetable Proteins

THANKS to the researches of Osborne, a number of vegetable proteins may be obtained in a pure state and in quantity. The work done in my laboratory was upon some of the seed proteins, especially zein from cornmeal and edestin from hemp seed, which were prepared by Leach according to the methods of Osborne. From these proteins we split off the protein poison by the same process employed in the cleavage of the bacterial proteins. The poisons obtained from zein and edestin showed no difference either in response to chemical tests or in physiological action from those obtained from the cellular substance of bacteria. My present purpose in bringing out these facts lies in the evidence, which they bear in support of my contention that the protein poison is a group in the protein molecule and that it is present in all true proteins. So long as my studies were confined to the highly complex bacterial proteins, I was not sure of the correctness of this idea. With edestin we are supposed to have an unmixed protein. It is a single compound, of highly complex structure it is true, but not a mixture of different molecules. If the poison be detached from this by chemical cleavage it must follow that the poison consists of a group which exists within the larger body. The importance of this will be more evident when I call attention to the fact that some years ago Pick and Spiro were unable to obtain from edestin the substance which when injected into animals retards the coagulation of the blood and from this failure they concluded that this body is not a true cleavage product of proteins and that it is not an intramolecular constituent of pure proteins. In fact they came to the conclusion that the coagulation-retarding substance is neither a protein nor a protein derivative. The relation between the protein poison and the coagulation-retarding substance will be discussed later. At this point I simply

*The Herter Lectures for 1916 given in the University and Bellevue Medical School, New York.

wish to emphasize my claim that the protein poison is an intramolecular constituent of proteins and that it is obtained by the chemical cleavage of protein molecules. Edestin being a simpler and smaller molecule than bacterial cellular substance is the more suitable matrix from which the protein poison may be obtained. The yield is larger and the by-products less in variety and abundance. Edestin contains no carbohydrate group while bacterial proteins contain two and these gave us much trouble in our earlier attempts to isolate the poison.

ANIMAL PROTEINS.

We have prepared the protein poison from a great number and variety of animal proteins, such as egg-white, casein, serum albumin, serum globulin, blood cells, muscle, brain, liver, kidney, etc. In fact, we have found no true protein which does not yield the poison when split up by the method given—a two per cent solution of sodium or potassium hydroxide in absolute alcohol.

In beginning this work I expected to find the simplest proteins in unicellular organisms. As I have already indicated this expectation has not been realized. The proteins of most simple structure I have found in seeds and in the casein of milk. Seeds contain the embryo accompanied by simple proteins and varying amounts of fat and carbohydrate, also proteolytic, amylolytic and lipolytic ferments. When the seeds are placed under proper conditions of temperature and moisture the ferments begin to act, the storehouses of foods are split into available building blocks and growth begins. In milk the food supply for the young is supplied in similar form. The carbohydrate exists in the form of milk sugar. The fat exists as such. The protein, in the form of casein, supplies the amino acids and the mineral substances are found mostly in the ash. The ferments are furnished by the digestive organs of the young. Digestion is relatively simple and easy, absorption proceeds quickly and growth follows.

Bacteria, although unicellular and simple morphologically, are made up chemically of highly complex molecules. There may be unicellular organisms composed of simple proteins but this certainly is not true of the bacteria which I have studied. In their chemical composition and structure these bacteria are quite as complex as the most highly developed cells in the animal body. It follows, therefore, that when we speak of bacteria as low and primitive forms of life, we should bear in mind that we are speaking as morphologists and not as chemists. Many, probably all, of the soluble proteins in man's body are chemically of much simpler structure than are those of the bacterial cell.

THE PROTEOSES.

Schmidt-Mulheim in trying to discover the fate of peptone in the blood (it being assumed at that time that peptones are absorbed as such into the blood) found that the intravenous injection of Witte's peptone, after the removal of the undigested proteins from this commercial preparation, caused in dogs striking physiological effects. The most notable among these were: (1) an inhibiting action on the coagulation of the blood and (2) a rapid and marked reduction in blood pressure. This work done in Ludwig's laboratory was continued a year later by Fano. Furthermore, it was shown that a second injection of peptone made shortly after recovery from the effects of the first had but little effect.

From these observations it became customary to speak of "peptone poisoning" and "peptone immunity." Fano did not confine his work to Witte's peptone, but made his own product by the digestion of fibrin with pepsin and trypsin. He also used Grüber's preparation and one from America. Grosjean used propeptone and peptone prepared by the method of Kühne and Chittenden and found that the former had a marked effect, especially when employed in doses of more than 0.15 g. per kilo. Arthus and Huber employed caseoses prepared by pancreatic digestion. Chittenden, Mendel and McDermott and later Chittenden, Mendel and Henderson produced highly poisonous bodies by breaking up proteins with a vegetable ferment papain, also with superheated steam and dilute acid without the aid of any ferment. Moreover, they found that all the primary, digestive protein derivatives have more or less marked effect upon blood coagulation and blood pressure. Pick and Spiro were unable to obtain a poisonous derivative from pure proteins, edestin and casein, and concluded that the poisonous agent present in mixed bodies is not a protein at all but an enzyme for which they proposed the name peptozyme. According to their view this in the proenzyme stage is widely distributed in the animal body, since they found the poison among the cleavage and digestive products of many organs. It might get mixed with the protein split products in digestion with an animal ferment, as pepsin or trypsin, or it exists in the tissue or protein which undergoes digestion; but take a pure protein like edestin or casein, and split it with acid and no poisonous body results. They claim that the poison never results from the hydrolysis of proteins with alkali. This is interesting in view of the fact that we have found cleavage with dilute alkali the best way of obtaining the protein poison.

Underhill has shown the incorrectness of the claim of Pick and Spiro and demonstrated that the proteoses are in and of themselves poisonous, when administered intravenously. He prepared the poison from pure proteins by cleavage with acids and showed that native proteoses found in seeds and nuts, wheat embryo, hemp seed and Brazil nuts, when introduced into animal intravenously induce all the symptoms formerly known as those of peptone poisoning.

Popielski has worked with a body which he extracts from commercial peptone with alcohol. This "vasodilatin," as he calls it, has the same action that was formerly attributed to peptone and notwithstanding its solubility in alcohol it gives the protein color tests, at least the biuret and the Millon.

It must be evident that the behavior of my protein poison both chemically and physiologically, closely resembles that of the proteoses. Some proteoses, at least, are soluble in alcohol, and as has been said, Popielski extracts his body from commercial peptones with alcohol. The protein poison, though soluble in absolute alcohol, gives the protein reactions and is a biuret body; some proteoses behave in a similar manner. Edmunds has shown that the protein poison lowers blood pressure in dogs, just as the "peptone poison" does. Edmunds did not find that the protein poison inhibits the coagulation of blood, but Underhill has recently showed that it has this effect, when used in larger doses than those employed by Edmunds. Underhill has recently compared the action of the protein poison with that of the proteoses and finds that the resemblance is strong

both in the effect upon blood pressure and coagulation, but "Vaughan's preparation differs from the proteoses in that it produces marked symptoms or even death in the rabbit in relatively small doses." The rabbit is mentioned here because of its known refractoriness to proteoses.

It seems to me highly probable that the poisonous group in the proteoses is the protein poison and that its more powerful action is due to the fact that it has been more effectually stripped of those groups which tend to neutralize its effects. It is present in every true protein and when molecular disruption proceeds up to a certain point the physiological action is increased, beyond that point it is decreased. The protein poison kills dogs, as shown by Underhill, in doses in which the proteoses have only a temporary effect, but the symptoms are the same. From this I conclude that the poisonous group is the same in both instances, but the free poison is more effective than the combined. This belief is confirmed by the fact that the free poison is easily split out of the proteoses by proper chemical agents.

THE AUTOLYTIC CLEAVAGE OF PROTEINS.

All proteins sooner or later undergo autolytic cleavage. When a solution or suspension of protein in water or salt solution is protected from bacterial invasion by chloroform or toluol and kept at about 37° the protein undergoes spontaneous cleavage. Salkowski seems to have been the first to investigate this phenomenon scientifically. This work has been continued by Biondi, Schwiening, Launoy, Jacobi and others. Most of these have given attention to cellular autolysis, as this is the most interesting phase of the subject, but all proteins, whether cellular or without structure, go through a similar process. Fibrin undergoes autolysis quite as promptly as liver cells do. It is well known that in multicellular animals proteases are generally distributed. At first it was assumed that these consist of the alimentary ferments which have been absorbed and distributed through the body. However, research has shown that the autolytic ferments differ from either pepsin or trypsin. In the first place they are possessed of a degree of specificity not characteristic of the alimentary enzymes. The ferment found in each organ or each kind of tissue digests especially, more rapidly and completely, the organ or tissue in which it is found. The liver ferment readily splits up liver tissue but is less effective in its action on the proteins of other organs. In the second place, the products of autolytic cleavage differ from those of enteral digestion. Pepsin forms large amounts of primary cleavage products, such as proteoses and peptons. These, especially the former, are highly poisonous, and would have a most disastrous effect were they liberated parenterally. The autolytic enzymes produce none or only traces of these primary split products. They cleave deeper and their chief products are the relatively harmless amino acids and purin bodies. From tryptic digestion the autolytic enzymes differ in several particulars. Trypsin acts in feebly alkaline solution while autolysis proceeds most rapidly in slightly acid media. It is more than probable that the intracellular tissue is always feebly acid. Tryptophan, a product of tryptic digestion, is seldom or never found among the autolytic products. In autolytic cleavage of proteins much more ammonia is found than in tryptic digestion. Furthermore the autolytic enzymes persist in animals from which the pancreas

has been removed. We see from these facts that protein tissues disintegrate normally in the animal body without the formation of poisonous products. It must be admitted that in certain pathological conditions, such as acute yellow atrophy of the liver and in phosphorus poisoning, autolysis proceeds with harmful rapidity and becomes at least a highly destructive process.

It has been suggested that the autolytic enzymes are constituents of the blood and are generally distributed through the body by this fluid. In other words it has been held that they are blood ferments. That this is not true is shown by the fact that blood and blood serum have an inhibiting effect upon autolytic action. Besides, proteins which contain no blood, such as egg-white, undergo autolytic cleavage.

The study of autolytic cleavage is complicated by the presence in many proteins of other ferments such as nucleases, arginases, etc. What effects the autolytic enzymes have upon foreign proteins is a question of importance, but one which cannot be answered at present. It will be understood that I have been speaking so far of the autolytic enzymes of the cellular and other proteins of the multicellular animal. When we come to speak of the autolytic cleavage of unicellular organisms, such as bacteria, we have quite a different problem. That bacteria undergo autolytic cleavage and that the products formed in this process may be harmful to multicellular organisms has been abundantly shown. Old cultures of colon and typhoid bacilli may contain soluble split products which are highly harmful and indeed may be fatally effective in their action on the higher animals. Whether pathogenic bacteria undergo autolytic cleavage in the bodies of their hosts is a question which, so far as I know, has not been decisively determined by experiment. The presumption is that this may and does happen.

The following experimental data concerning the autolytic cleavage of bacterial proteins may be of interest in this connection:

Rosenow has shown that pneumococci suspended in salt solution and kept at 37° for forty-eight hours, under ether or over chloroform, undergo autolysis with the liberation of a poison. This poison injected intravenously or intracardially in normal animals induces anaphylactic shock. In guinea-pigs death results from bronchial spasm and consequent arrest of respiration. In dogs it causes marked fall in blood pressure and delays the coagulation of the blood.

I took powdered pneumococcus cellular substance which had been prepared nearly seven years before. Microscopic examination showed the pneumococci as clearly defined and in as perfect form as in a fresh preparation. Five hundred milligrams of this powder was suspended in 500 c.c. of salt solution, 10 c.c. of chloroform added and kept at 37°. After twenty-four hours 10 c.c. of the opalescent supernatant fluid was administered to a guinea-pig intravenously. Within two hours the animal's temperature fell to 94°, but recovery followed. The same experiment repeated after 48 and 72 hours killed the animal within two hours with the symptoms of subacute anaphylactic shock. A like injection after six days killed within three minutes with all the symptoms and postmortem findings of acute anaphylactic shock.

It has been shown that the cholera bacillus does not undergo ready autolytic cleavage in vitro, but there is reason for suspecting that this happens in the intestine of infected men, since after death the bacillus is found only in the

intestinal canal, in some instances at least, all the internal organs being sterile.

Warden finds that the gonococcus early undergoes autolysis and that the autolysates are fatal to guinea-pigs. He believes that the autolysis of this organism is not due to enzyme, but results from a disruption caused by the absorption of water by the cells.

PARENTERAL PROTEIN DIGESTION.

We now distinguish between enteral and parenteral digestion. We take diverse proteins into our alimentary canals and through the activity of the enteral digestive ferments they are split into amino acids which are utilized by the body cells in growth and in function. This is the normal way in which the body cells of the higher animals are fed, for the most part at least. Under normal conditions the amount of protein reaching the blood and lymph undigested is small and negligible in effect. Minute bits of unbroken protein may find their way into the circulation through the respiratory and digestive tracts. These, entering through the respiratory organs, may cause local sensitization which manifests itself in the complex of symptoms usually designated as hay—rose— or horse-fever and asthma. Those passing in undigested forms through the walls of the alimentary canal may lead to the untoward effects of certain articles of diet and possibly may exert a more serious action on some of the more distant organs, especially the kidneys.

During fetal life all the food enters the body parenterally and there is no enteral digestion. There are reasons for suspecting that during infancy the chief milk protein, casein, may be absorbed in part in an unbroken state. At least in a few instances unchanged casein has been detected by the biological test in the blood of infants suffering from summer diarrhea.

In my opinion there are reasons for believing that in some animals a certain part, or certain kinds, of protein food are absorbed unbroken and are digested parenterally. Rabbits are easily sensitized, notably by casein fed by the mouth or administered by the rectum. I have detected the protein in the heart's blood by the biological test after such feedings. While there is a promising field for research along these lines, it is safe to say that in man in health, the amount of unbroken, foreign protein reaching the circulation is small. Protein in appreciable quantities reaches the blood only when injected, as in the employment of sera and vaccines or through infection. In the latter instance the protein multiplies in the body.

It is evident that one or more of the following effects may result from the parenteral introduction of a foreign protein: (1) It may be eliminated through the kidneys. (2) It may be passed into the alimentary canal and there digested. (3) It may be digested parenterally. All these dispositions may be employed in the disposal of the foreign protein.

The literature concerning the renal elimination of foreign proteins is voluminous, but often contradictory. The occurrence and extent of this form of disposal vary with the kind of protein, the quantity, the rapidity of introduction, the species and individuality of the animal and probably upon many unknown conditions. It was formerly supposed that all the protein passing through the kidneys after parenteral introduction consists of that introduced. It has been

definitely shown that this is not true and the estimates found in the older literature showing the per cent eliminated by the kidney are without value. Some years ago it was shown in my laboratory that in the urine of rabbits after the parenteral introduction of egg-white, both egg-white and blood protein appear. Guinea-pigs were sensitized to both with the urine. This gives no indication of the proportion in which they were present." It has been shown by Chiray and confirmed in my laboratory that foreign protein injected into the blood soon disappears from the circulating fluid and carries with it an appreciable amount of the proteins of the blood. So far as I know Chiray is the only one who has made frequent observations of the effects of the parenteral administration of proteins in man. He frequently induced albuminuria in this way, especially in those who already showed renal inefficiency. In rabbits he induced marked structural changes in the kidneys by repeated injections.

In my work on the parenteral introduction of proteins, I have carefully controlled the rate of injection and have found that the foreign protein is more likely to appear in the urine when the rate of injection is high. When the protein is slowly introduced, I have been surprised at the large amount that can be introduced into the abdominal cavity or into an ear vein without any detectable trace appearing in the urine.

When heterologous proteins are injected into the blood they soon find their way into the intestinal lumen. They are poured in with the bile and they pass into the abdominal cavity and through the intestinal walls. With the biological test we have detected proteins injected into the ear veins of rabbits in the liver, abdominal cavity and lumen of the intestines. It seems to be a general physiological law that poisons introduced into the blood are eliminated in part at least into the alimentary canal. Morphine given subcutaneously may be detected in washings from the stomach. Gastric erosion may be induced by the subcutaneous or intravenous administration of arsenical preparations. So long ago as 1753 Sproegel showed that gastric lesions may be due to arsenic absorbed from wounds, and since that time they have been induced in animals by the hypodermic administration of neutral solutions of arsenic. Similar lesions are seen in poisoning with antimony and other metals and may result in these instances also from application made to wounds and to raw surfaces. Mercury when employed by inunction is poured into the alimentary canal and its destructive action may be seen in almost any part from the mouth to the rectum. Erosions of the stomach and intestine may be extensive and deep, even to perforation. The fact that gastric and duodenal ulcers may follow severe burns of the skin has been long known and is best explained by supposing them due to the large amount of poison resulting from the burn, being brought to the walls of the alimentary canal. The gastric inflammations and erosions of the acute infectious diseases are doubtlessly due to the same cause. The smallpox virus has a predilection for epithelial tissues and manifests its destructive action in the skin and in mucous membranes. It has long been known that peptic ulcer is frequently associated with chronic appendicitis and the recent brilliant work of Rosenow has called attention to the probable relation between peptic ulcer and pyorrhea. In case of a nidus of infection in any part of the body poisonous proteins are being poured into the circulation and these like other poisons are carried to the walls

of the intestine for the evident purpose of elimination. Here they accumulate and in their reaction with the body cells, the latter are more or less injured. The elimination of proteins from the blood into the alimentary canal holds for both living and dead, formed and unformed proteins. This is an interesting phase in the study of the action of poisonous proteins and is worthy of further study.

It has been long known that blood serum, like living cells, is highly resistant to proteolytic enzymes. Furthermore the presence of blood serum markedly retards both peptic and pancreatic digestion. It has been generally inferred from these facts that blood serum contains an antiproteolytic ferment and since the reaction is alkaline, this is generally designated as antitrypsin. So far as I know, Camus and Gley were the first to show experimentally that blood serum inhibits peptic and tryptic action. These investigators observed that fibrin or coagulated egg-white placed in serum and treated with active pepsin or trypsin remains intact. More extended observations have shown that many, if not all, kinds of proteolytic digestion, are retarded, often wholly arrested, by the presence of blood serum. There is another interesting fact in this connection. The injection of proteolytic ferments into an animal, especially repeated injections, increase the potency of the blood-serum in the inhibition of the action of that ferment. Antibodies are formed and accumulate in the blood after repeated injections of pepsin, trypsin, rennin, etc. The effect of such injections is similar, probably closely related, to that which follows injections of toxins. But little is known concerning these antibodies in case of either the ferments or the toxins.

Delezenne and Pozerski first showed that chloroform removes from blood serum the antiproteolytic body. They found that blood serum has no digestive action on gelatin under ordinary conditions, but that blood serum which has been extracted with chloroform promptly digests gelatin. The researches of Jobling and others have confirmed and amplified this work and it has been shown that when the unsaturated fatty acids are removed from blood serum by extraction with chloroform or ether, it becomes highly poisonous even for the species from which it was derived. Whether Jobling is right in his contention that the fatty acids constitute the antibody is still to be determined. It is possible that the extraction of blood serum with chloroform may have some effect upon the equilibrium in its protein constituents.

Friedberger found that the blood serum of the guinea-pig when incubated with bacterial cell substance becomes poisonous. He explained this on the assumption that the proteases of the serum digest the bacterial cells with the formation of a poison which he calls anaphylatoxin. Later it was shown that the guinea-pigs serum when incubated with agar or starch becomes poisonous. From these findings it was suspected that bacillary substance, agar and starch, act upon guinea-pigs by absorption of the antibodies. In this way the proteases in the serum are relieved of the presence of their antibodies and digest the proteins in the serum. In other words, the matrix of the poison consists of the proteins in the serum and not of the bacillary cell substance.

Abderhalden found that when placental tissue is digested with the serum of pregnant women diffusible digestive products are formed and may be detected in the diffusate by the biuret and ninhydrin tests. He explained this by sup-

posing that placental tissue in small amount finds its way into the maternal blood, and that this fluid acquires the property of digesting placental proteins. Abderhalden believes this to be a specific reaction and has proposed it as a diagnostic test for pregnancy. This test has been studied by many and while its significance cannot be considered as finally settled the weight of evidence seems to be that Abderhalden's explanation is not correct. It seems from the evidence now at hand that the placental tissue absorbs the anti-ferments and the unopposed protease of the serum digests the protein constituents of this fluid.

The weight of evidence today discards the idea of specific proteases in blood serum and favors the idea that certain antibodies exist in the serum and when these are reduced in amount, the non-specific protease of the blood serum acts upon its own protein constituents. It must be admitted that this view is more in accordance with some of the facts than the one which holds that specific proteases are existent in the blood or may be brought into existence. However, it should be stated that the present view does not exclude the necessity of regarding protein digestion in the blood as, in some instances at least, specific. Take the production of anaphylactic shock as an example. The theory proposed by Wheeler and me in 1907 supposes that when a given protein is first injected parenterally into an animal, it slowly develops a specific protease. This is a cellular product. Certain cells stimulated by contact and by penetration with the foreign protein develop a new, specific protease which is capable of digesting that protein and no other. The protein of the first injection is disposed of by this new specific ferment, but is broken up so slowly that no harm comes to the animal, or at least no recognizable danger, from the cleavage products. The cells continue in the possession of the newly acquired function. This may persist for years and indeed throughout life. The animal is said to be sensitized. On reinjection of the same protein the body cells, having acquired the function of digesting it, do so with such violence that the digestive products endanger the life of the animal or at least develop physiological disturbances which are easily recognizable. We have offered this in explanation of the success of vaccination. The vaccine virus is introduced into the child's arm. The proteins of which the virus is composed are distributed in the body and sensitize certain cells. This means that the cells develop a ferment which destroys the vaccine virus and the new function developed in these cells by their first experience with the smallpox protein in its attenuated form continues in the possession of the cells for years. When the vaccinated person is exposed to smallpox the virus of the disease is destroyed before it has time to multiply and consequently the individual is protected from the disease. Please understand that I am not ready to give up the theory of the formation of specific proteases. I see no other explanation of the immunity conferred by vaccination or by one attack of the disease. However, in presenting this matter I wish to proceed without being influenced by preconceived ideas, and I wish to repeat that the idea of a non-specific protein digestion in anaphylactic shock especially has much in its favor, both in fact and in theory. The poison developed in anaphylactic shock may not come from the protein of the reinjection and the protease developed in sensitization may not be specific. Anaphylactic shock may be due wholly to the unmasking of a non-specific ferment and the poison formed may come from the proteins of the blood, but if all

this be true, and the weight of evidence today is in this direction, the anaphylactic reaction remains specific. We have only transferred the problem of specificity from the development of a specific enzyme to the specific uncovering of a non-specific enzyme. It remains true that an animal sensitized to one protein is not sensitized to other and unlike proteins.

I have said that the theory of the uncovering of a general protease in anaphylactic shock has much in its favor. The blood seems to be a fluid in which ferments and anti-ferments are nicely and delicately balanced and a slight disturbance in this equilibrium leads to marked effect. We have obtained from one gram of casein enough of the protein poison to kill 800 guinea-pigs when injected intravenously. That casein, the chief protein constituent of the food of all mammalian young, should be found to contain a body so highly poisonous when introduced intravenously is certainly a surprising thing. However, the surprise does not disappear when we go further and find that a similar poison may be obtained not only from all the proteins we eat but also from those that make up the tissue of our own bodies. Indeed, every gram of protein in an animal's body may supply enough poison to kill many such animals. There are other interesting things about this protein poison besides its potency. When amounts of it, even smaller than the minimum lethal dose, are incubated with blood serum *in vitro*, the serum, in itself inert, becomes fatally poisonous. In these studies a curious phenomenon has been observed. The incubating serum containing the poison may be fatally active at the expiration of a given time, then later wholly without effect, and later still fatally active. This wave of appearing, disappearing, reappearing toxicity we have frequently observed. For it I have not even the shadow of an explanation. It may turn out after all that ferments and anti-ferments are not concerned in these phenomena. I have tried to think of oscillations induced in a colloidal fluid like the blood serum by the presence of the protein poison, but I have not been able to fix such a concept.

As was first shown by Friedberger bacterial cellular substance incubated with blood serum *in vitro* renders the serum poisonous. In repeating these experiments and injecting the serum at intervals, at one time it kills with all the violence of anaphylactic shock, then it has no effect, then again it kills. I have tried to time this wave of toxicity, but adjust every condition to the best of my ability, I have been unable to chart it. It is to be hoped that some wiser man with more perfect control of the conditions of his experiments will solve this question. I am willing to leave it to those braver than I to try on human beings such poisonous mixtures of bacterial proteins as phylacogen.

I have stated that I am not yet ready to give up the idea that the parenteral introduction of foreign proteins produces specific alterations in the blood. I cannot do so so long as I have the evidence supplied by the specificity of agglutination and precipitin reactions. We may have no proof that these are due to the development of specific proteases, but whatever their action it is within certain limits specific. Some years ago with my assistants I published the results of work which I interpreted as demonstrating the elaboration of specific proteases in sensitized animals. The results were all so clean cut and uniform that they were convincing to me at least. I will give a brief abstract: (1) One mg. of egg-white incubated at 37° for thirty minutes in 5c.c. of the serum or organ extract of unsensitized guinea-pigs is without effect when injected into the heart

of another unsensitized guinea-pig. (2) Like results followed when the incubation was made with fluids obtained from an animal sensitized three days previously with egg-white. (3) When the fluids were obtained from animals sensitized fourteen days previously to egg-white anaphylactic shock followed in all. (4) With the conditions as in (3) except that the incubation was prolonged to ninety minutes the effects were less marked. (5) With the conditions the same as in (3) except that the incubation was done in a cold room the effects were nil. (6) When the fluids were obtained from an animal seventeen days after sensitization anaphylactic shock resulted. (7) Filtration of the fluids through hard fiber paper did not affect the results. (8) Filtration of the serum and organ extract through a Berkefeld V did not affect the results. (9) Filtration after incubation did not affect the results. (10) When the serum and organ extracts were heated to 56° for thirty minutes there were no effects. (11) When the heated serum and organ extract were activated by the addition of unheated serum and extracts the effects were positive. (12) When the serum and organ extracts of a guinea-pig sensitized to egg-white were incubated with horse serum there were no effects. (13) The serum and organ extracts of guinea-pigs sensitized to horse serum elaborated a poison when incubated with horse serum. (14) The serum and organ extracts of guinea-pigs sensitized to typhoid bacilli gave a poison when incubated with typhoid bacilli. (15) Like results were obtained with the cholera bacillus. (16) In case of egg-white the serum ceases to be active in about forty days after sensitization. (17) When the amount of portein incubated with the serum and organ extracts was larger than 1 mg. per 5 c.c. the results were less certain.

If there was not more luck than science in these experiments they clearly show specificity. I now know that there would be a chance of getting some positive results with a non-specific serum, but it seems impossible for these results to have been so uniform on any other ground than that of specificity. Besides, they compare with the results obtained by Pfeiffer who found that the sera of guinea-pigs digested, for about forty days after sensitization, the protein to which the animal had been sensitized.

Abderhalden and his students in numerous experiments have shown by the polariscope that the blood serum of a sensitized animal has a more marked digestive action on the specific anaphylactogen than has the serum of a non-sensitized animal. Similar results have been obtained by dialysis methods by Pfeiffer and Mita, by Pfeiffer and Jarisch and by Zunz and György. The last mentioned have apparently shown a marked increase in amino-acids during anaphylactic shock. This controverts the finding of Auer and Van Slyke.

I have repeatedly found, as others have, that the blood serum shows sensitization for relatively a short time, while the animal remains in a sensitized condition much longer. This observation has convinced me that protein sensitization is accompanied by and is due, in some instances at least, to a profound and lasting impression made on the cells of the body. Indeed, there can be no doubt that protein sensitization is cellular. Pearce and Eisenbrey bled a sensitized dog into a fresh one and at the same time replaced the blood taken from the sensitized one by that of a second fresh one. The sensitized dog from which all its blood had been removed responded with anaphylactic shock on reinjection, while the dog now carrying all the blood of the sensitized one did not.

(To be continued.)

THE PERIPHERAL ACTION OF OPIUM ALKALOIDS WITH SPECIAL REFERENCE TO THE BLADDER*

BY D. E. JACKSON, PH.D., M.D., ST. LOUIS.

IN an article¹ published in 1914 I have shown that a considerable number of the alkaloids of opium when injected intravenously in large doses into spinal dogs cause a profound and exceedingly persistent broncho-constriction. In the present article I wish to discuss a series of results which I have recently obtained in dogs on another organ, viz., the bladder.

There are two series of opium alkaloids, the first including morphine (and its derivatives, such as dionine, heroine, peronine), codeine, thebaine, etc., and the second including narcotine, papaverine, narceine, laudanine, and a considerable number of others. The members of the first group are derived from phenanthrene ($C_{14}H_{10}$), while those of the second group are derivatives of isoquinoline. Altogether there are some twenty-five separate alkaloids present in crude opium.

In the present experiments I have dealt mainly with alkaloids of the first group (phenanthrene), but by comparing the present results with those formerly obtained I have been able to draw certain inferences regarding the action of the second group.

METHODS.

Generally spinal dogs have been used in these experiments, but in some cases animals with the central nervous system entirely intact have been used as controls. The animals were always etherized and arranged for blood-pressure, bladder and (usually) lung tracings. To secure the bladder tracings an incision was made in the abdomen over the bladder which was drawn up a little and an opening was made in the fundus. Into this opening a small tube was tied, the upper end of the tube being connected by a short piece of rubber tubing to the lower end of the opening of a glass mercury bulb (250 c.c.). A perforated cork, through which passed a glass tube, was placed in the neck of the mercury bulb. The glass tube was connected by rubber tubing to a large bowled recording tambour which wrote on a smoked drum. If on opening the abdomen the bladder was found to be distended, care was used to avoid its being emptied while the recording apparatus was being attached. A bladder which is thus allowed to become emptied out during manipulations sometimes passes into a state of contraction which is difficult to overcome. Urination was prevented by clamping the penis or vulva with a hemostat. When all adjustments for recording were made then the mercury bulb was partly filled with warm salt solution and the cork was inserted. The record was thus obtained by both liquid and air transmission. The abdomen was carefully closed with hemostats and arrangements were made for recording the lung changes.

The lung records were usually made by means of a special piece of apparatus which I described² in detail some years ago, and which when fitted into the

*From the department of pharmacology of Washington University Medical School, St. Louis, Mo.

thorax through a median incision in the sternum holds the chest wall rigidly distended and practically air tight. Respiration is then carried on by intermittently aspirating air out of the chest cavity. A small adjustable by-pass permits more air to enter the chest between aspirations. But when the air is thus forcibly sucked out of the chest air will enter the lungs through the trachea and thus inspiration occurs. In the intervals between aspirations the lungs collapse from their own elasticity and thus expiration is effected. A large bowled recording tambour is attached to the side tube of the tracheal cannula. This gives a measure of the amount of air entering (down stroke) the lungs in inspiration or leaving (up stroke) in expiration. Contraction of the bronchioles decreases the amount of air passing into and out of the lungs and thus the amplitude of the record on the drum is decreased. Dilation of the bronchioles increases the amplitude (height) of the tracing.

Blood pressure was recorded with a mercury manometer from the right carotid artery. Usually the brain and cord down to the thoracic region was destroyed in pithing and in a few instances for a special purpose the cord was destroyed throughout its entire length. Injections were made through the femorals or external jugular veins.

In a few preliminary class experiments I discovered that certain members of the series of opium alkaloids might produce marked contraction of the bladder. I have therefore in these experiments extended these early observations and attempted to get some insight into the extent and origin of these reactions.

The literature on opium extends back to the time of Theophrastus, Scribonius Largus and Dioscorides and is so exceedingly extensive that one can scarcely hope to see more than a relatively small number of the published articles. There are numerous references to the marked increase of intestinal peristalsis³ which morphine, etc., may produce in some animals such as the dog. In man constipation is the rule. I have not, however, found any significant references bearing on the observations which I have made in the present experiments. In some excellent papers published in 1915, Lieb and McWhorter,⁴ Barbour and Copenhagen,⁵ and Barbour⁶ alone have discussed the action of morphine on the gall bladder (excised), and on the uterus (excised and in situ). These observers all noted that under some conditions morphine might produce either a feeble contraction or a slight increase in tonus of gall bladder and of uterine muscle. In some experiments, however, relaxation or a decrease of tone was seen.

Fig. 1 shows the result of injecting 25 milligrams of morphine into a 6.5 kilo dog. The bronchioles contract vigorously and the bladder contracts (up stroke) to a less degree. This is a relative matter, however, and the extent of contraction of the bladder varies with different animals and with the condition (amount of distension) present when the drug is injected. While morphine here causes only a small contraction of the bladder, in other experiments extensive contraction may be seen. This holds good for all of the phenanthrene derivatives which I have examined. Injection of 1-2 c.c. of adrenaline (1-10,000) causes a dilation of the bronchioles but produces scarcely any change in the bladder. In this experiment the bladder was probably almost empty at

the time of injection of the morphine, and in a rather strong tonus. This dog had been etherized and pithed, but had not received any other medication.

Fig. 2 was obtained by injecting 25 milligrams of codeine through the femoral vein. Both bladder and bronchioles contract vigorously and synchronously. Evidently these contractions are strictly analogous and the rate and period of relaxation in general correspond fairly closely, sometimes one and sometimes the other gaining the upper hand. Injection of "epinine" (1 2 c.c., 1 c.c., 2 c.c.) causes only a slight change (dilatation) in the lungs and probably has but little effect on the bladder. The blood pressure does not rise much, chiefly because of the asphyxia from the broncho-constriction.

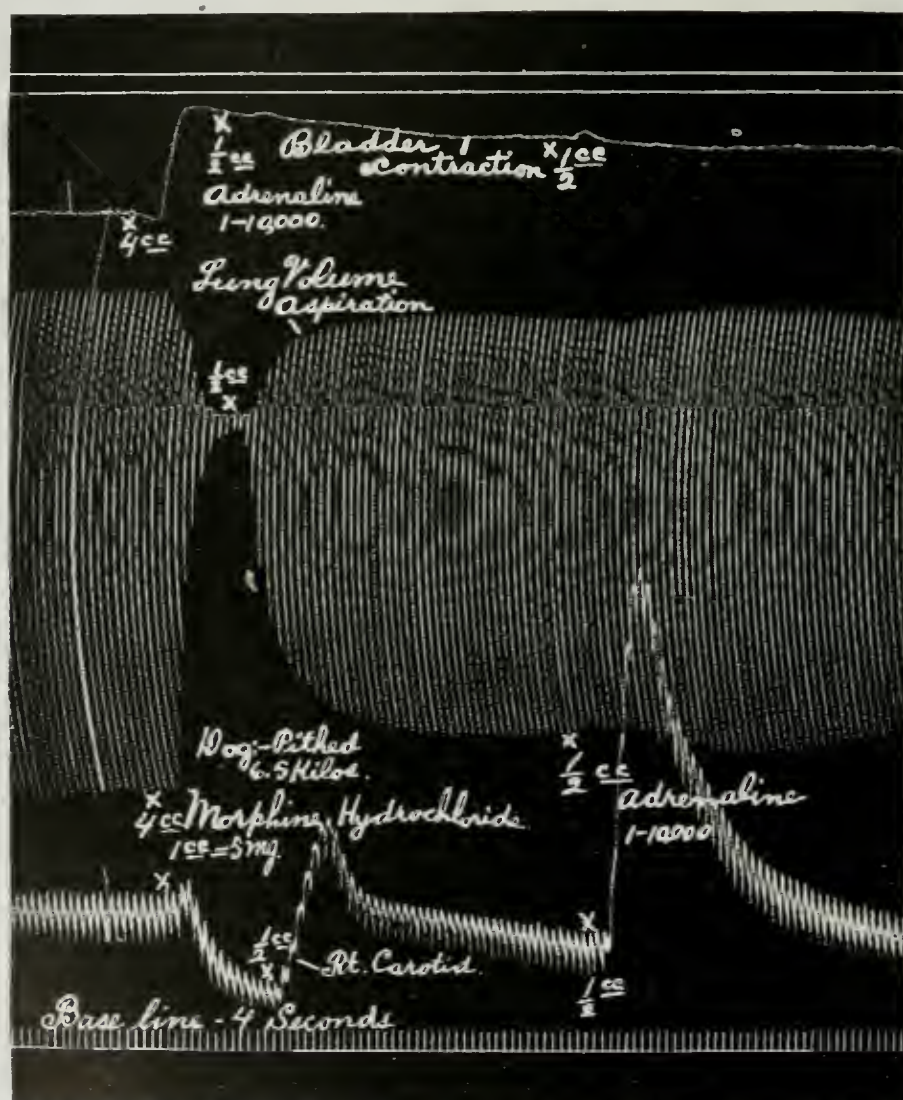


Fig. 1.—For description, see text.

Fig. 3 shows the action of 30 milligrams of thebaine on an 18 kilo dog. The bladder contraction is very marked and injection of adrenaline (1 c.c. of 1-10,000) causes dilation of the bronchioles, but in the case of the bladder a second contraction is produced. This action of adrenaline is variable, the record sometimes showing a contraction and at others a dilation of the bladder. Apparently this depends partly on the condition and amount of distension present in the organ when the adrenaline is injected. If the bladder is almost empty and in tonus, then a slight dilation is usually but not always produced.

while if the organ is distended, then adrenaline as a rule will cause some contraction. But the results, while sometimes rather striking, are very variable. This perhaps depends on the innervation of the organ partly, but probably slight disturbances in the bladder circulation, and the amount of any previously

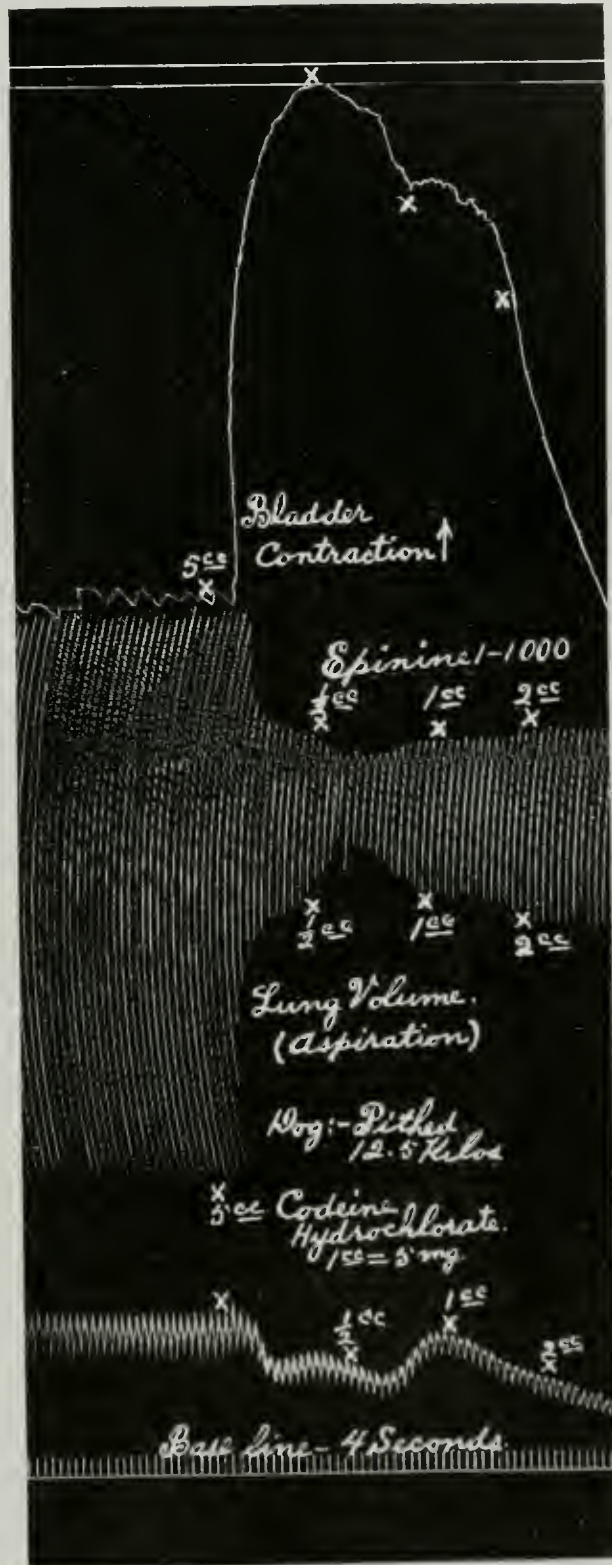


Fig. 2.

injected drug which may still remain in the tissues of the bladder, have something to do with it. The action of the adrenaline here should be compared with that shown in Fig. 4, in which 25 milligrams of heroine were injected into an (different animal) 18 kilo dog. This caused a marked bladder (and bron-

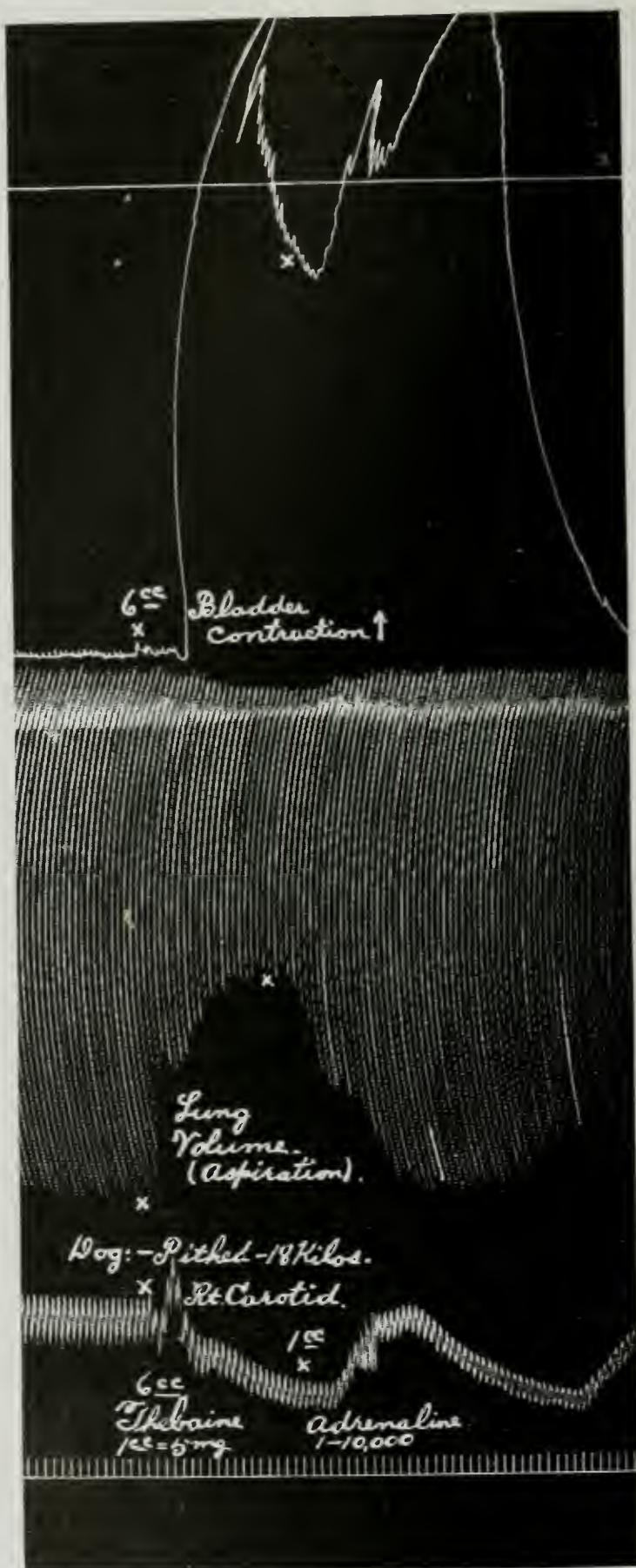


Fig. 3.

chiole) contraction. Three injections of adrenaline are shown following this. In each case these apparently caused some dilation of the bladder (the first dilation being shown at the top of the initial contraction curve).

A new point may now be taken up. In Fig. 4 it will be seen that the first

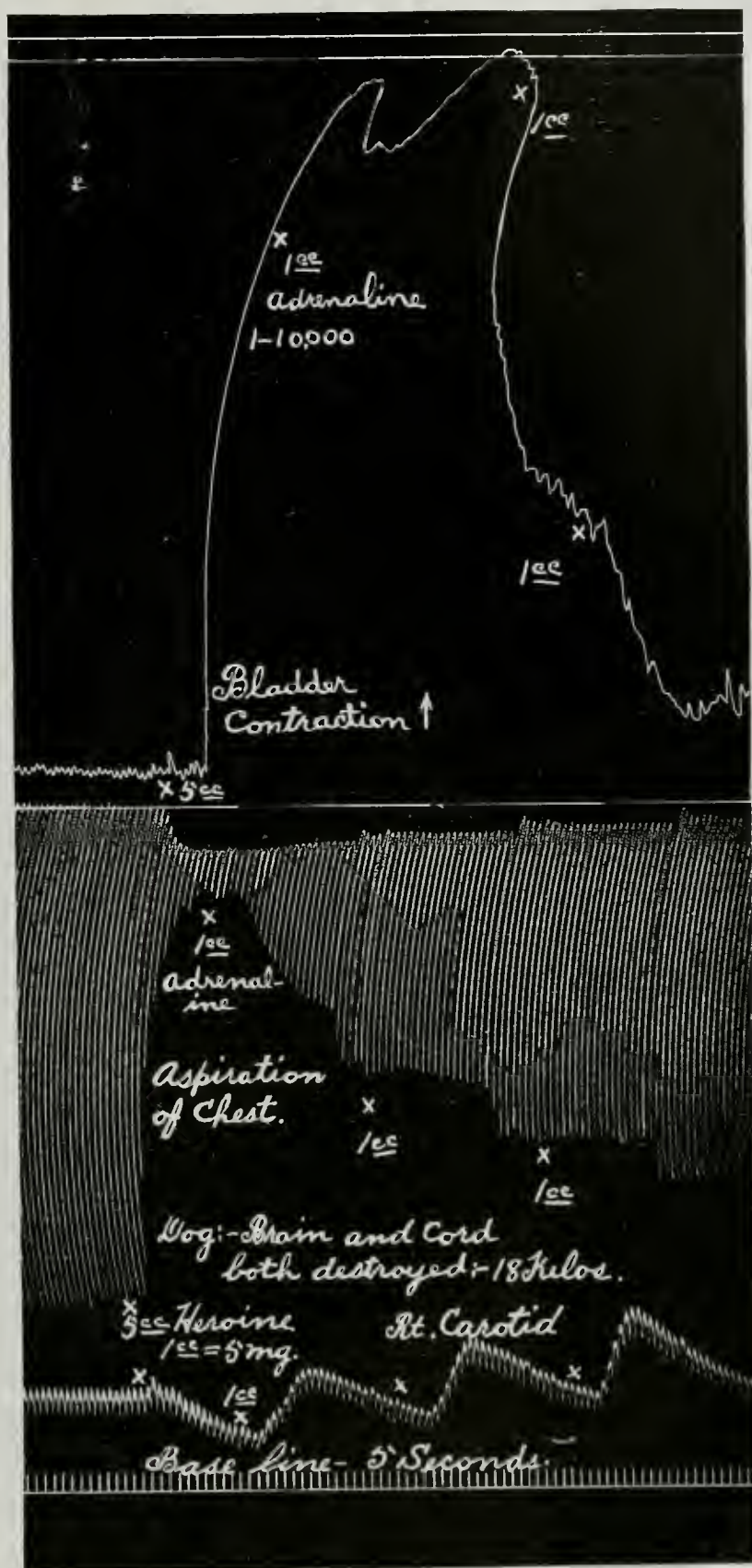


Fig. 4.

injection (5 c.c. = 25 mg.) of heroine caused a marked contraction of both bladder and bronchioles. This was followed by three injections (1 c.c. each) of adrenaline. Following the dilatation of each of these, further injections of heroine were given. Fig. 5 shows the results of the second, third and fourth of these injections. In these injections 7 c.c. (35 mg.), 10 c.c. (50 mg.) and 5 c.c. (25 mg.) of heroine were given. The 7 c.c. injection being considerably larger

than the initial 5 c.c. dose we would expect to get a correspondingly larger bladder and bronchiole contraction. And this result might be expected to be even more marked with the 10 c.c. injection. On the contrary these very large doses produce practically no effect whatever on the bladder and only a very moderate slow contraction of the bronchioles. The effect on the heart (blood pressure) is also correspondingly very much less than that produced by the

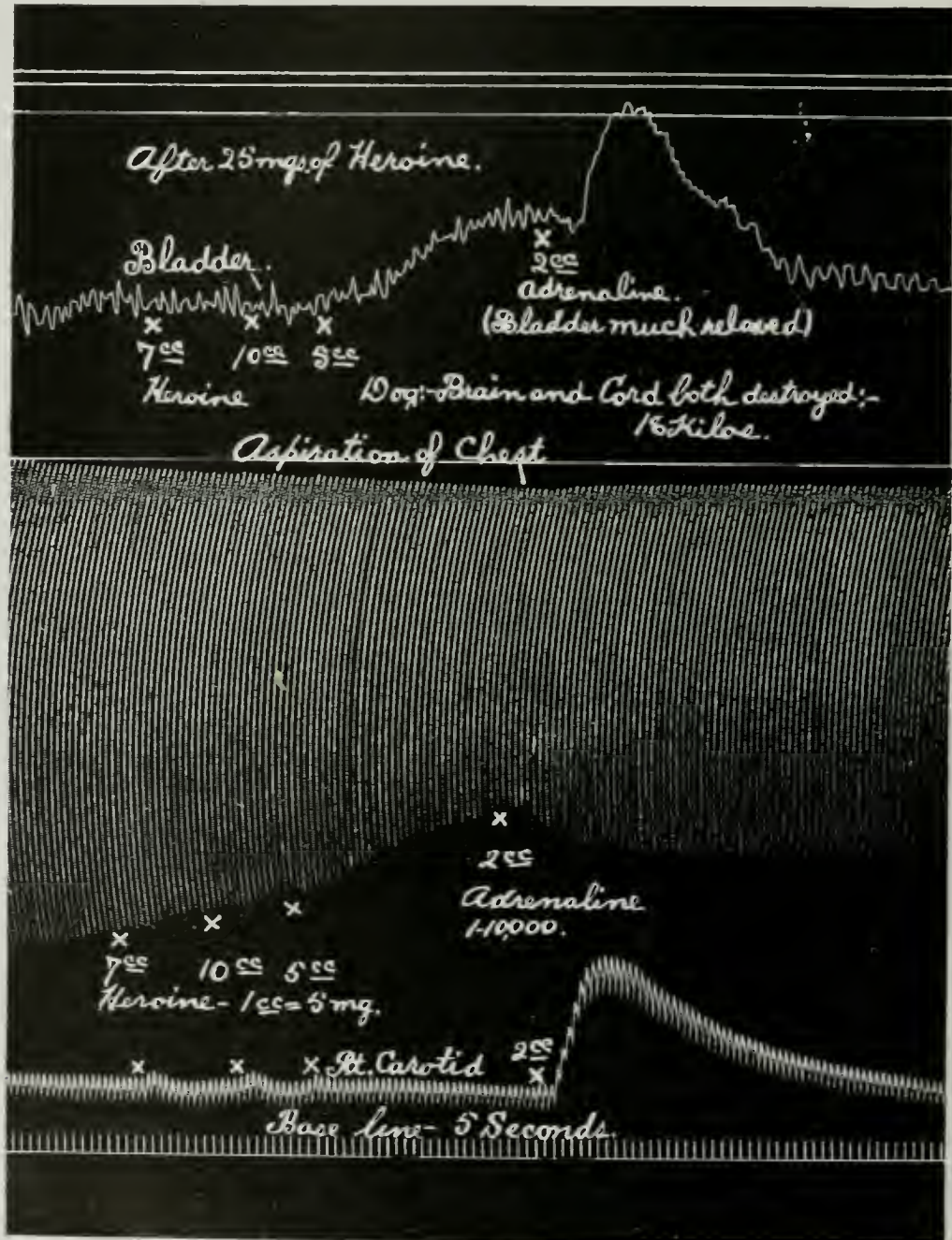


Fig. 5.—Made directly after Fig. 4.

initial smaller dose. These results appear to be perfectly constant for all of the phenanthrene derivatives and possibly for a few of the other series, especially narcotine. It is also found that this failure of the bronchioles and bladder to respond well to a second injection of the drug holds good in the case of the bronchioles for any other member of the opium alkaloids, i.e., in the case here given in which the first contraction was due to heroine, then a second injection of morphine, or thebaine, or peronine, etc., will not cause a second contrac-

tion, provided the initial contraction was maximal. If the first contraction was small (dose submaximal) then the injection of a second much larger dose may

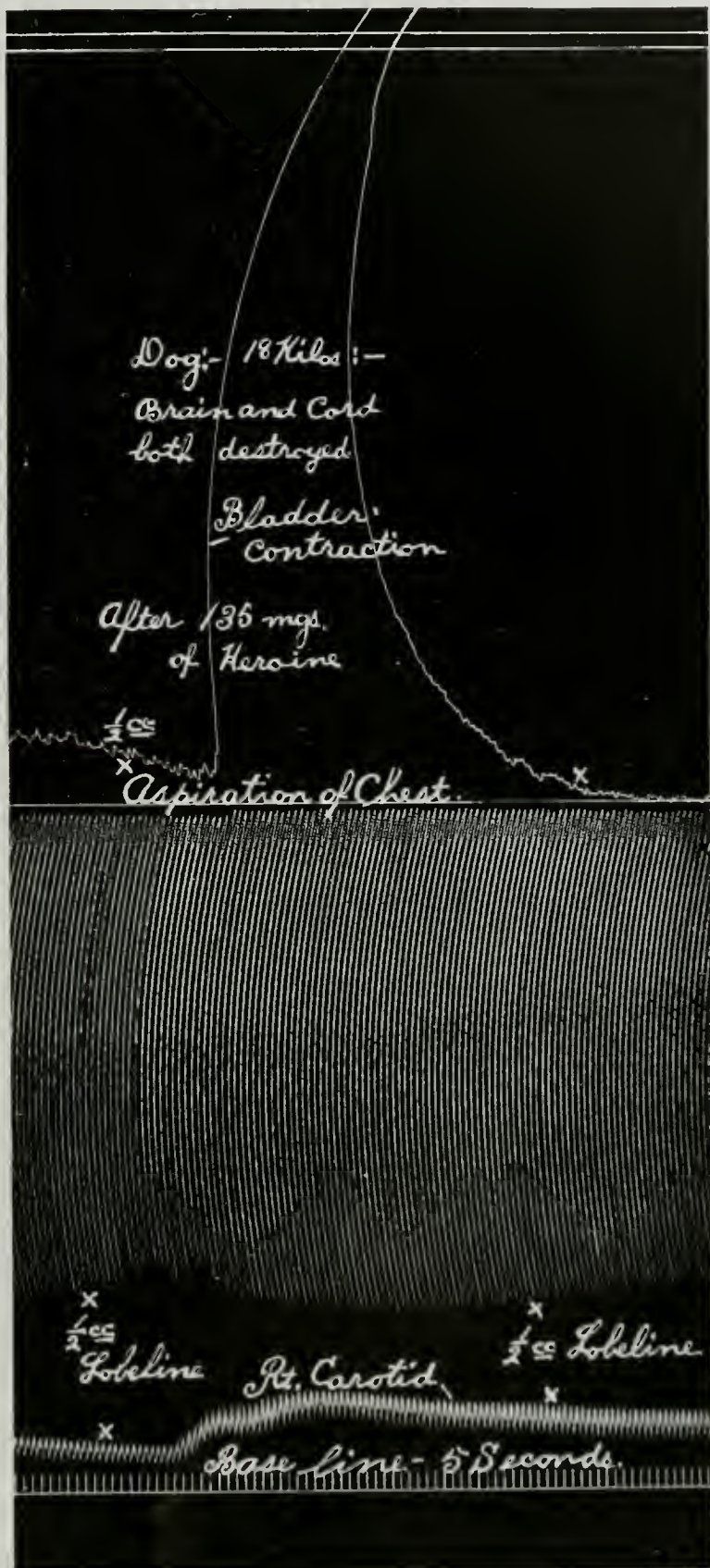


Fig. 6. Made directly after Fig. 5.

give a small contraction. But even in this case a third injection of any sized dose very generally gives no contraction of either bladder or bronchioles. I observed this point long ago and mentioned it in the previous paper on the

slow for the blood pressure is low.) This shows that not only the musculature, but even the innervation of the bladder was practically normal so far as one can tell from the lobeline reaction. A second injection of 1/2 c.c. of lobeline produces no results at all, showing that complete ganglionic paralysis followed the stimulation produced by the first dose of the drug. I should em-

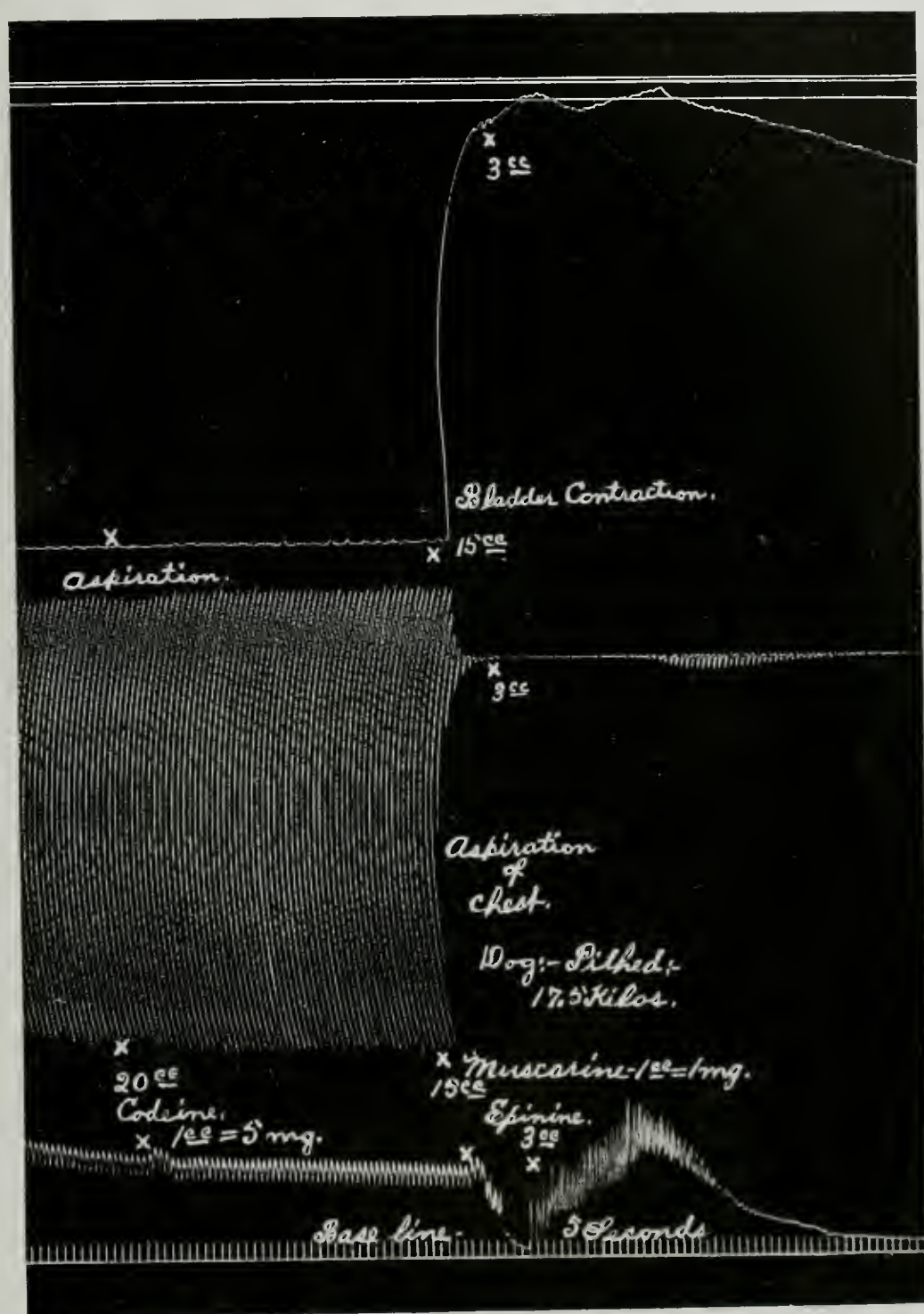


Fig. 8.—Made directly after Fig. 7.

phasize in passing that not only the brain but also the entire spinal cord was destroyed in this animal.

The question naturally arises as to where these drugs act. Since in Fig. 6 a marked contraction of both bladder and bronchioles was obtained in a dog with the entire central nervous system destroyed, one must next proceed to examine the peripheral structures. Fig. 7 shows the results obtained in an experi-

ment in which in a pithed dog 1.2 c.c. of lobeline was first injected. This gave a marked reaction both of the bladder and of the blood pressure. As soon as both of these returned to normal another 1.2 c.c. of lobeline was injected. This produced no effect whatever, showing that the ganglia on the sympathetic (autonomic) nerves were all paralyzed. Following this 6 c.c. (30 mg.) of codeine hydrochlorate were injected. A prompt contraction of the bladder and a small shrinkage in the air volume followed. Shortly after this 2 c.c. of "epinine" were given. This caused a rise in blood-pressure and a faint contraction of the bladder. A little later 12 c.c. (twice the former dose) of codeine were given and a small contraction of both bladder and bronchioles was produced. In this case the initial contraction from codeine was not maximal. Consequently, a second (double) dose gave about one-half as marked an effect on the bladder. (The lungs were contracted more than one would expect, but the initial contraction of these was small.) This was followed by injections of "epinine" to dilate the bronchioles. Directly after Fig. 7 was produced a large dose (20 c.c.) of codeine was given (Fig. 8) and no effect whatever was obtained on either bladder or bronchioles. Evidently the first two injections caused a complete loss of sensibility of both bladder and bronchioles to codeine. One would suspect this might be due to a depression or paralysis of the motor nerve (sacral autonomic) endings in the bladder or of those in the bronchial muscles. If codeine should first stimulate and then paralyze these endings, the above records might very well be explained. This action, however, does not occur, for in Fig. 8, directly after the 20 c.c. of codeine, 15 milligrams of muscarine were injected and a prompt contraction of both bladder and bronchioles followed. Muscarine stimulates the endings of the constrictor nerves in the bladder and bronchioles and hence these could not have been paralyzed by the codeine. Death resulted from asphyxia due to contraction of the bronchioles, for the 3 c.c. of "epinine" given were not sufficient to cause a bronchiole dilation. The animal could easily have been saved by forcibly dilating the bronchioles (by increasing the force of aspiration from the chest).

Another item of some interest should be noted in Figs. 7 and 8. In the first of these 6 c.c. of codeine produced a considerable fall in blood pressure and probably some slowing of the heart. After this effect had mainly passed off, however, the 20 c.c. given in Fig. 8 caused only a slight rise from the increase of intravascular volume of fluid and practically no fall in pressure at all. Thus the vascular system as well as the bladder and bronchioles has lost its sensitivity to the codeine. This is a regular occurrence although it is not so striking as is the loss of action on the bladder or bronchioles.

In Fig. 9 is shown a tracing obtained from a spinal dog which had received in small repeated doses intravenously 25 c.c. of a saturated solution of curare (Kahlbaum's). Here injection of 5 c.c. (25 mg.) of heroine caused only a small contraction of the bladder and no effect at all on the lungs. The blood pressure, however, fell about as usual. Following this 1/4 c.c. of adrenaline (1-10,000) was given. The effect of this can be seen on both the bladder and the blood-pressure. A little later 1/2 c.c. (1/2 mg.) of arecoline was injected and caused a fairly marked contraction of both bladder and bronchioles (by stimulating the constrictor nerve endings in these organs). The pur-

pose of this injection was to control the conditions of the experiment and of the animal, i.e., to determine if the apparatus and tissues are all responding in a normal manner. A later injection of 1/2 c.c. of adrenaline was given to restore the animal.

From this experiment one would be inclined to suspect that curare has some counteracting influence to the action of the heroine. While the contrac-

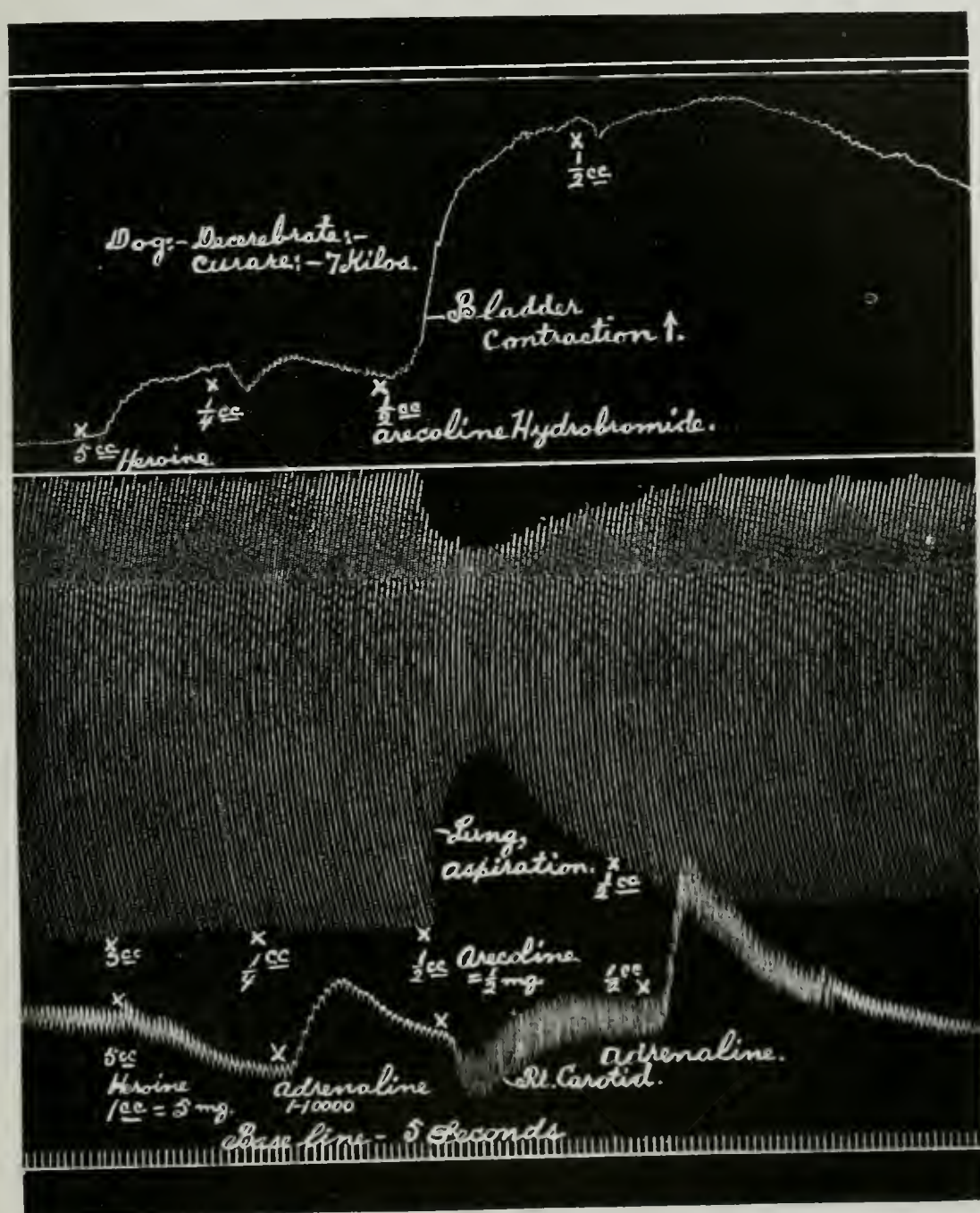


Fig. 9.

tion is not large, it still is present, however, and the curare has certainly not completely paralyzed the structures on which the heroine acts.

Fig. 10 shows a modification of this experiment in which a pithed curarized dog in which a complete ganglionic paralysis had been produced by lobeline, received an injection of 5 c.c. (25 mg.) of heroine. A fairly marked contraction of both bladder and bronchioles is produced. A later injection of 1-2 c.c. of adrenaline dilated the bronchioles but contracted the bladder. This dog had

received intravenously $18\frac{1}{2}$ c.c. of curare solution in small repeated doses. While curare cannot prevent heroine from causing a contraction of the bladder, still my impression is that the curare in large doses, may very materially reduce the effects of heroine. I believe this will hold for other drugs of the series also, but I have not tried these out.

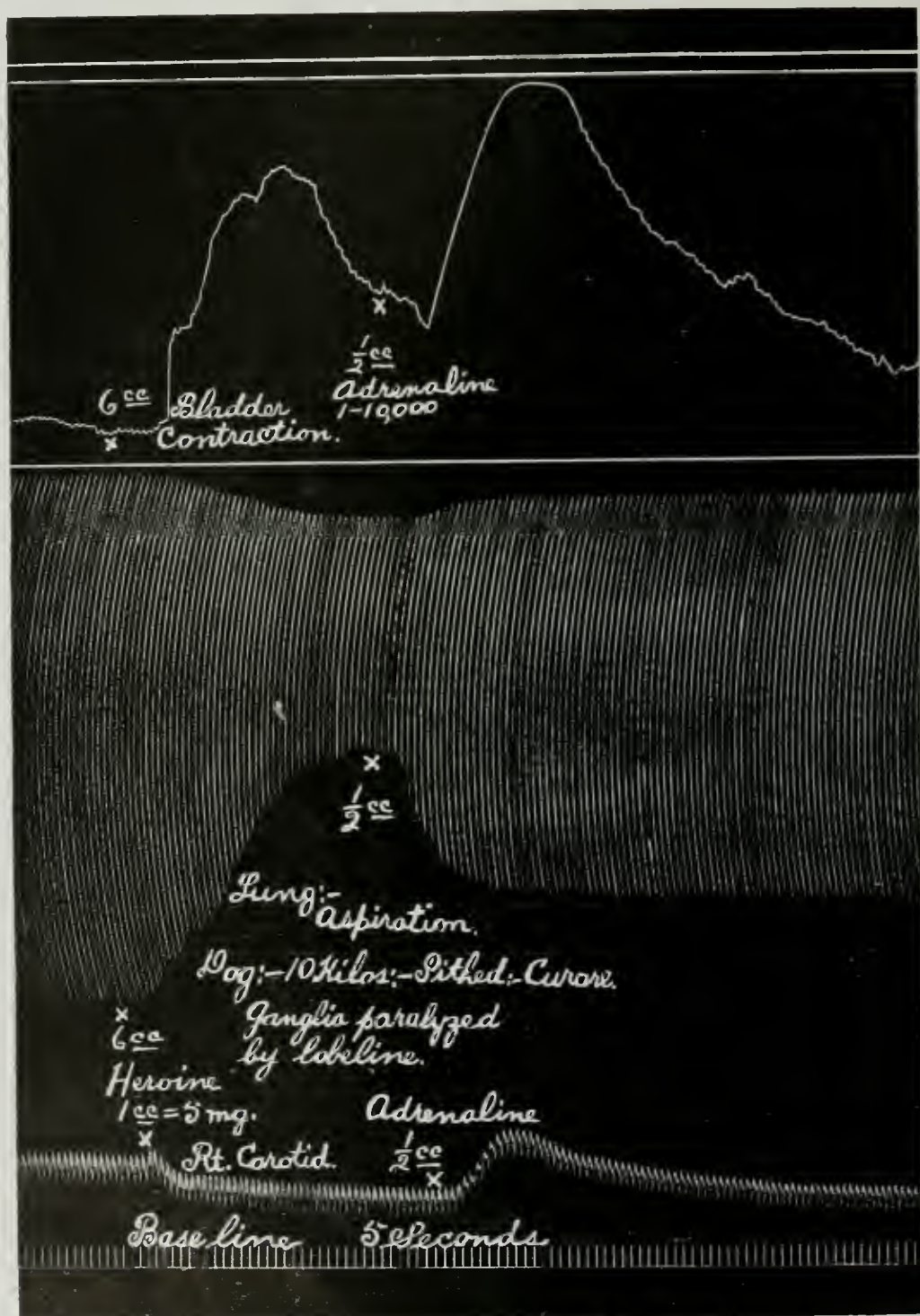


Fig. 10.

Fig. 11 shows in a spinal dog, which had received 3 milligrams of atropine, the effect of injecting $\frac{1}{4}$ c.c. of lobeline solution. A marked contraction of the bladder and arterioles (rise in blood-pressure) followed. A second injection of $\frac{1}{4}$ c.c. of lobeline produces about half as much effect and is followed by ganglionic paralysis as is shown by a later injection of $\frac{1}{4}$ c.c. of lobeline,

which produces no effect at all on the bladder and circulation. Following this, 5 c.c. (25 mg.) of heroine were injected and a prompt contraction of both blad-

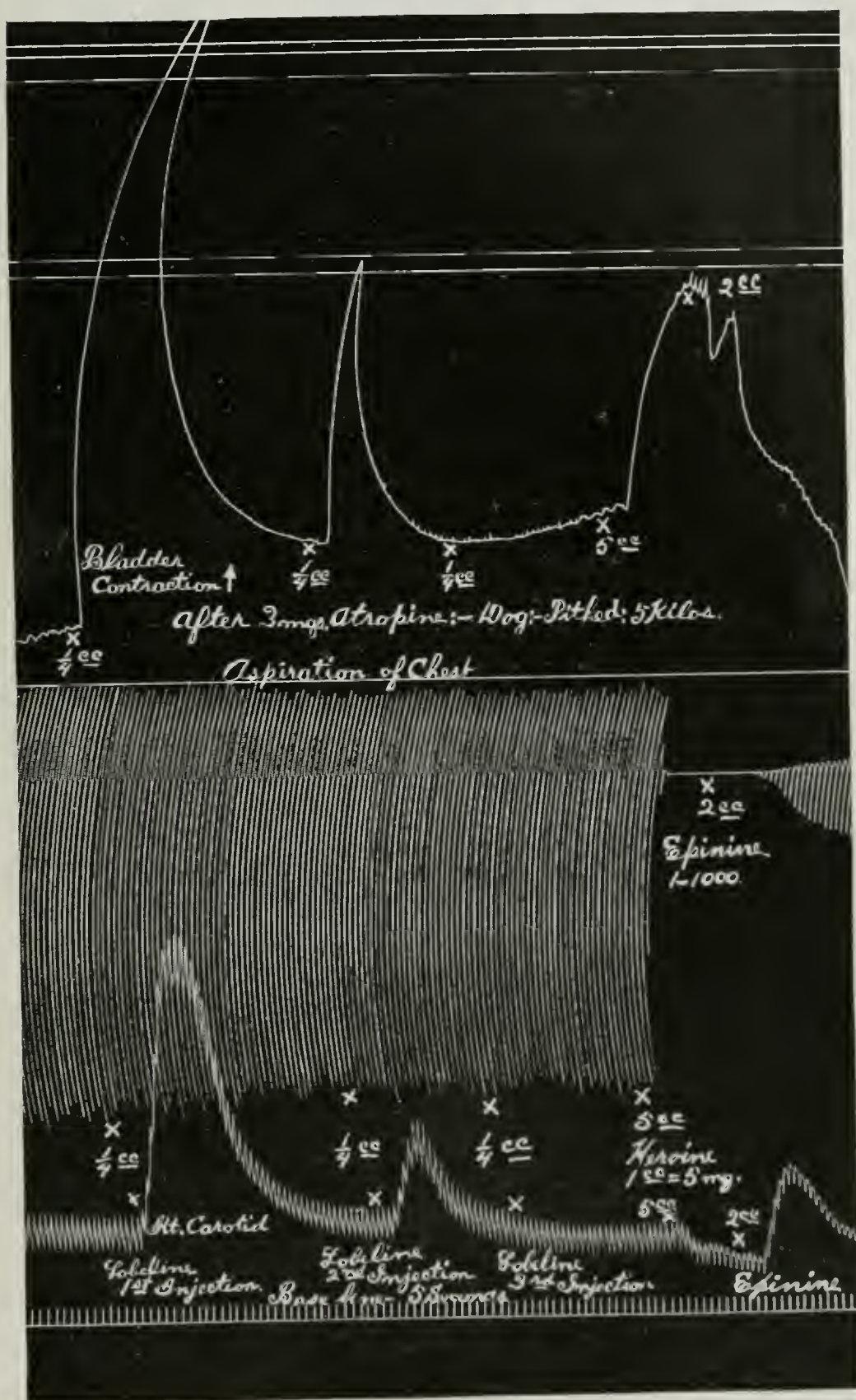


Fig. 11.

der and bronchioles followed. This shows that lobeline and atropine (3 mg.) were not sufficient to prevent the action of the heroine. A later injection of "epinine" was given to revive the animal (by dilating the bronchioles).

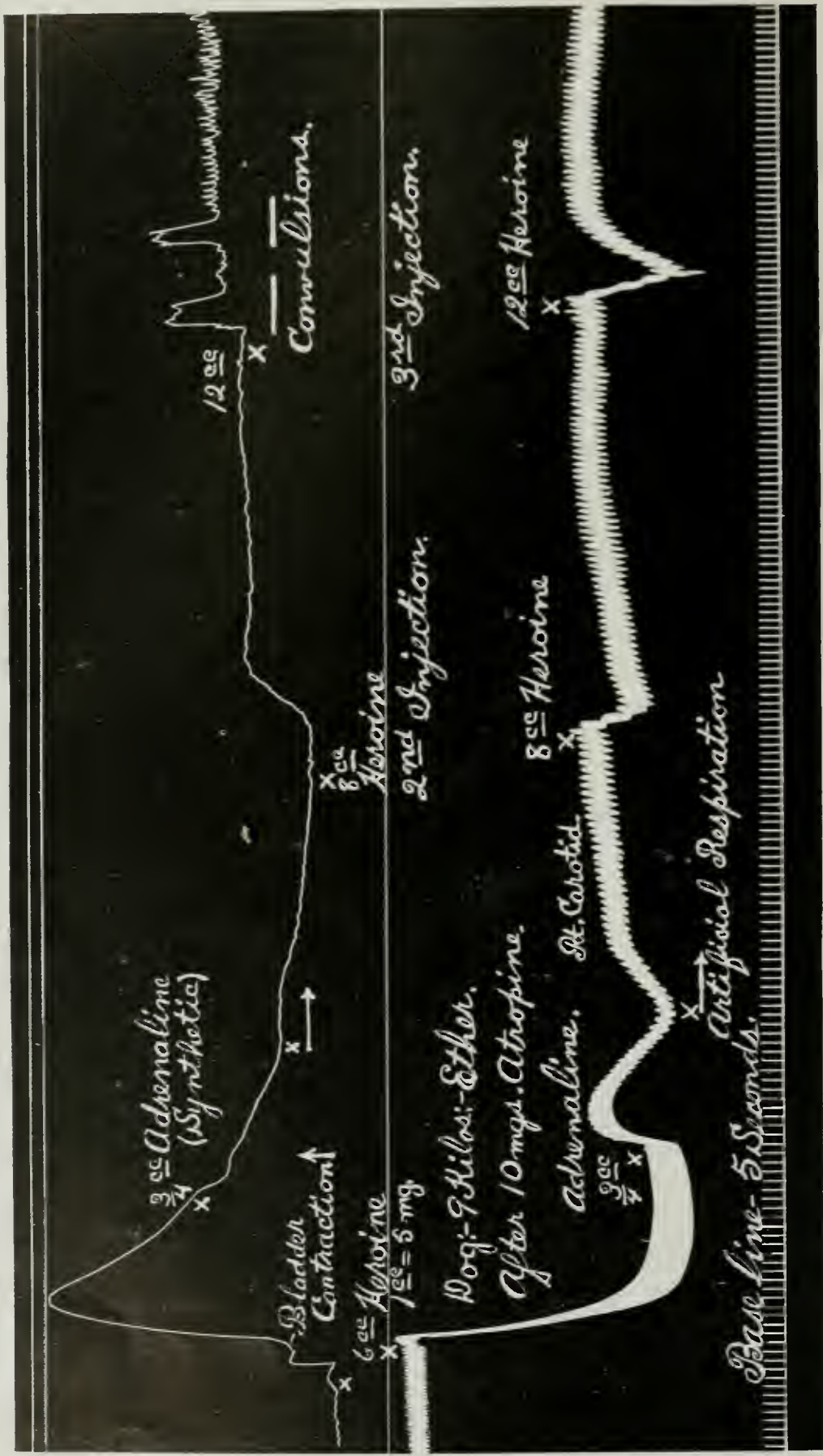


Fig. 12.

Fig. 12 was obtained from a 9 kilo dog after 10 milligrams of atropine (in small repeated doses) had been administered intravenously. A well marked contraction of the bladder occurs when 6 c.c. (30 mg.) of heroine is injected.

This animal was not pithed and was under the influence of ether. This, undoubtedly indicates that these opium alkaloids act directly on the muscle fibers of the bladder. A little later $3/4$ c.c. of adrenaline was injected which caused only a slightly increased rate of relaxation of the bladder. The respiration ceased and at the point indicated artificial respiration was begun. A second injection of 8 c.c. of heroine gave a small bladder contraction which persisted throughout the rest of the tracing. After some time 12 c.c. of heroine (twice the initial dose) were injected. This brought on a few moderate strychnine-like convulsions but caused no contraction of the bladder. The two small rises in the bladder record were caused by these convulsions. They were carefully observed in order to be certain that the bladder records as obtained in the whole series of experiments were not due to compression of the bladder from contraction of the abdominal muscles. There is no resemblance between the convulsion records and those obtained when no convulsive tremors whatever were produced as is very generally the case especially with the first injection. The curare experiment and those with both brain and cord destroyed also further rule out any confusion between the records obtained and those which might be produced by convulsions.

SUMMARY.

The experiments herein described prove that those opium alkaloids which belong to the phenanthrene series, i.e., morphine, codeine, thebaine, heroine, dionine, peronine, etc., in large intravenous doses cause in dogs a profound contraction of the bladder. I have previously shown that a similar contraction of the bronchioles is produced by these drugs. Probably a few of the isoquinoline opium alkaloids, including narcotine, may also cause a similar action. I have failed in a few experiments to obtain this contraction with some other ones of these alkaloids notably papaverine, cryptopine and cotarnine.

Apparently the contractions of the bladder and of the bronchioles produced by these drugs are strictly analogous and in all probability of identical origin. The contractions in both organs occur simultaneously, are usually of approximately the same proportions, last for corresponding periods of time, and when the initial contraction is maximal then later injections of any sized doses of either the initial drug or of any other of the series will not produce any further contraction whatever of either bladder or bronchioles.

If the initial contraction was not maximal then much larger doses of the initial drug or of another of the (phenanthrene) series may, *in much larger dose*, cause a second contraction, but even by proceeding by degrees in this manner, a third contraction is almost never obtained. When the bladder and bronchioles have thus lost their susceptibility to the action of these opium derivatives they are still found to possess practically normal sensitivity to all drugs which usually act on them including lobeline, nicotine, pilocarpine, arecoline, muscarine, atropine, barium, vanadium, adrenaline, etc. Previous destruction of the brain and spinal cord or injection of lobeline or atropine or both does not prevent or probably even specifically decrease the extent of the reaction to heroine, codeine, morphine, etc. Possibly curare in very large

doses may weaken the response of the bladder and bronchioles to these opium bodies.

These reactions closely resemble those produced by drugs which first stimulate and secondarily paralyze nervous structures such, for example, as the action of lobeline on ganglia. But so far as I have been able to determine by pharmacological means *no paralysis of either nervous or muscular structures* is produced by these alkaloids. Ordinarily we would be inclined to attribute this action of the opium bodies to a direct action on the muscle fibers, since it occurs after atropine (and curare). But since a bladder which has become completely immune to further injections of these opium bodies may give, so far as I have been able to determine, a perfectly normal response, i.e., a profound contraction, to ordinary doses of barium, vanadium, pilocarpine, muscarine, or even lobeline, I am unable to see why the loss of response to the opium alkaloids should be attributed to muscular rather than to nervous origin.

Throughout the course of these experiments I have been much impressed with the similarity which a number of the reactions produced by these alkaloids bear to certain phenomena produced in anaphylactic shock. For in some animals, such as the guinea pig, death is produced in this condition by bronchial spasm which is in all probability very similar indeed to that produced by these alkaloids. And Dale⁷ has shown that in the excised uterus of a sensitized guinea pig reactions to the sensitizing serum, or (in some instances) to other protein solutions, may be obtained which bear a striking similarity to the immunity to later doses which a bladder acquires by one initial maximal response to one of these opium alkaloids.

In consideration of the extensive use of morphine for experimental purposes it is interesting to note these very marked reactions in dogs to the drug. Especially is this true for the tolerance or loss of susceptibility to the action of the drug. For while in many of these experiments large doses have often been used, still the striking, initial, maximal contraction which caused a loss of response to the drug was usually obtained with about 25 or 30 milligrams ($\frac{1}{2}$ grain). This dose is very frequently equaled or exceeded in narcotizing dogs for experimental purposes. What effects these profound reactions may have on the experimental conditions, or on the reactions to a larger series of drugs than I have used, remains to be seen. In consideration of the great tolerance which the chronic opium user may attain for these bodies, especially morphine, the reactions here described acquire a special clinical interest. Myers⁸ has recently shown that in normal intact dogs a cross-tolerance for certain opium alkaloids (phenanthrene series) may exist between closely related drugs but that this tolerance in intact dogs is evidenced only on those functions on which the drugs have a common selective action.

In conclusion, and for the benefit of others who may sometime care to repeat some of these experiments, I want to especially emphasize a point which I have observed in a few rare instances. This consists in a complete failure to obtain any contraction of either the bladder or the bronchioles or both. Usually I have been inclined to attribute this to impure or deteriorated drugs or to poor technic, but the possibility that in those animals the peripheral structures,

such as the bladder or bronchioles, may have possessed a special resistance to the action these drugs (independently of the central nervous action) may be worth considering.

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ANTIDOTES IN MERCURIC CHLORIDE POISONING.—AN EXPERIMENTAL STUDY*

BY BERNARD FANTUS, M.D., CHICAGO, ILL.

THE wave of mercuric chloride poisoning, through which we passed during the last few years, has been followed by a wave of suggestions for new antidotes to corrosive mercuric chloride. It was for the purpose of testing the antidotal efficiency of these, as compared with that of albumen, that this research was undertaken.

The great uncertainty of interpretation of clinical results, coupled with the necessity of determining what antidote offers the patient the best chances for recovery, renders experimental work on this question actually imperative. The first requisite to such study is a reliable method; to the devising of which, for the purpose in hand, my first efforts were directed.

Because of the closer relationship of gastrointestinal functions of the dog to those of man, than is found in other laboratory animals, dogs were first used in an attempt to find a surely lethal dose for this animal. In this attempt I failed. For dogs vomit so easily and mercuric chloride is so irritating to the stomach, that it is next to impossible to obtain uniform results, even if care is taken to secure an empty stomach by withholding food; and even if the tendency to vomiting has been lessened by preliminary administration of morphine, and the poison is administered after the morphine emesis has taken place.

More uniform results were obtained with the rabbit, which animal was, for this reason, chosen in this investigation. This animal's inability to vomit insures retention of the dose. The disadvantages of using the rabbit are that, in the first place, the stomach of the rabbit is never empty. It requires prolonged starvation before emptiness of the stomach can be secured. The lesions in the stomach are, therefore, generally less severe than they would have been had the stomach been empty. The bowel of the rabbit is exceedingly slow in emptying itself. To determine how long it takes, in the rabbit, before ingested material has left the alimentary tract completely, a normal rabbit was given 4

*From the Department of Pharmacology and Therapeutics, College of Medicine, University of Illinois.

gm. of wood charcoal. The black color did not appear until the second day, and *persisted for eleven days*. This is probably five times longer than in the human. In view of this slow evacuation of ingested material, it is probably fair to assume that, if in our experiments a rabbit lived considerably longer than the usual lethal period, an antidotal effect had been obtained; an effect that might have been life-saving in an animal with more rapid evacuation of the bowel, e.g., man.

It was first of all necessary to determine a uniformly lethal dose of mercuric chloride for the rabbit, which was done by means of experiments, the summary of which is shown in Table 1.

TABLE 1
DETERMINATION OF LETHAL DOSE OF MERCURIC CHLORIDE ON STOMACH TUBE
ADMINISTRATION TO RABBITS

Dose per Kg.	Number of Experiments	Lived
0.01	1	30 days
0.02	1	4 days
0.03	1	7 days
0.04	9	3 days average
0.05	1	2 days
0.06	5	1 ² / ₅ days average

Showing that all these doses are lethal; the larger the dose, the prompter the death.

In all of these experiments, the mercuric chloride was administered in one per cent solution, by means of the stomach tube, to rabbits that had not been starved. It will be seen that the main difference between the effects of these various doses, is in the lethal period, i.e., the length of time that elapses before death takes place. Choosing 0.04 gm. per kg. as the smallest dose that was followed by a reasonably short lethal period, I performed a sufficient number of experiments (nine) to enable me to feel confident that it was a certainly fatal dose, and that the corresponding lethal period is not subject to great variation. The lethal periods in our experiments with 0.04 gm. per kg. were as follows: 2, 2, 2, 3, 3, 3, 3, 3, and 5 days: a variation certainly sufficiently small.

Necropsy was performed on all these animals, and it may be of interest to note that the animal which lived 5 days, and one of the three day animals, had only slight lesions in the stomach; all others showing marked or extensive corrosion. The two typical and constant lesions that were easily found on necropsy were: first, a necrosis of the tips of the folds in the cecum; and, secondly, changes in the kidney, which in rabbits dying within 3 days was usually a swelling and softening of the organ; its normal striations, especially those of the cortex, being obscure or entirely obliterated. In animals living a little longer, we often found a very marked abnormal striation in the cortex, yellowish white and reddish-brown streaks alternating with each other. Microscopic examination of such specimens showed extensive degeneration, especially of fatty type in streaks, separated from each other by areas showing mainly congestion and exudation. Occasionally, though rarely, a kidney would be obtained that had an almost uniform yellowish color, a specimen in which fatty degeneration had become general. Albumin, casts, and frequently red blood corpuscles were found in the urine of all the poisoned animals, in which urinary examination was made.

The effects of dilution were next obtained, for, in giving the antidote, it

is frequently necessary to introduce it with a considerable amount of water. The results of the experiments to determine this factor are given in Table 2, from which it will be seen that dilution up to 1:1,000 had no marked effect upon the lethal period, which was, for the seven observations, an average of three days, the same as obtained in the nine rabbits that were given the poison in one per cent solution. The main effect of dilution is to lessen the local lesions in the stomach, which in the animals that had received the poison in 1:1,000 solution were rather slight. In our subsequent work, an attempt was made not to exceed a dilution of 1:1,000.

To determine the value of an antidote, the first step I took was to administer it mixed with the poison. Obviously if the resulting mixture was found to be quite as toxic as the poison given alone, it was unnecessary to go further in the study of that substance. If, on the other hand, it was found that animals survived the fatal period by a considerable time, then the antidote was administered immediately after the poison had been injected into the stomach. If the substance proved of marked antidotal value when given immediately after the ad-

TABLE 2

INFLUENCE OF DILUTION UPON EFFECT OF LETHAL DOSE

Lethal Dose of 0.04 gm. per Kg. of Mercuric Chloride administered with varying quantities of water.

Rabbit	Weight	HgCl ₂	Water	% of Sol.	Died in	Necropsy Lesions	
						Local	General
20	910	0.036	3.6	1%	3 days	Marked	Marked
21	905	0.036	3.6	1%	2 "	"	"
22	900	0.036	7.2	1/2%	2 "	"	"
23	1140	0.046	9.2	1/2%	3 "	"	"
24	1225	0.049	19.6	1/4%	3 "	"	"
25	1500	0.060	60.0	1/10%	5 "	Slight	"
26	1355	0.054	54.0	1/10%	3 "	"	"

This table shows that dilution has no marked effect upon the lethal period. If dilution is considerable (1:1000) it lessens the severity of the local lesions.

ministration of the poison, then it was given 5, 10 and 15 minutes afterward, to see whether it is likely to be of use under the conditions prevailing in practice. The chief item to be noted in the estimation of results, is the period of survival of the animal after the poison was given. This is given in number of days, though the figure 100 was not exceeded. In other words, whenever an animal is reported to have lived 100 days, it means that it has survived that period; and it seems that such animal may be considered to have recovered. Some of these lived much longer. Some of those that lived over 100 days died later from inter-current diseases, e.g., rabbit pneumonia. Though a few of those that died after 100 days showed the lesions of chronic nephritis on necropsy, it seems fair to conclude that in view of the sluggishness of the rabbit's bowel, and the consequent prolonged retention of the poison in the system, if the kidney was damaged to so slight a degree as to permit the animal to live for three months, or even half that period, a really marked and valuable antidotal effect has been obtained, one that would have been probably life-saving in the human, and which might, therefore, properly be scored as a recovery.

ALBUMIN.

As is well known, albumin is the standard antidote for mercury. Hence, we may begin the report with the results obtained with that substance, or rather that group of substances, for under this name are included a large number of different bodies.

After preliminary test tube experiments, to determine the amount of albuminous fluid necessary to remove practically all free mercury ions from the solution, such amount was administered mixed with the poison. Having shown, for instance, that 1,000 times as much milk would practically remove one part of mercuric chloride from the solution, that amount was given mixed with the poison with the result shown in Table 3. The animals lived no longer than they would have lived, if water had been used as diluent. The chief difference was

TABLE 3
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) MILK

Rabbit	Weight	Mercuric Chloride		Milk	Lived	Necropsy Lesions	
						Local	General
G6	1900	0.076	w	76.00	4 days	0	Marked
G7	1270	0.051	w	51.00	3 "	0	"
G8	1210	0.048	w	48.00	2 "	0	"
G9	761	0.030	w	30.00	3 "	Slight	"

This table shows that milk is of no antidotal value in Mercuric Chloride poisoning. Animals lived an average of 3 days after the poisoning, which is the average lethal period, when no antidote is used.

that there were practically no local lesions, i.e., lesions in the stomach, on necropsy. Milk is therefore, text-books to the contrary notwithstanding, of no antidotal value in mercuric chloride poisoning in rabbits. It emphasizes, on the other hand, the value of milk in the oral administration of mercuric chloride for medicinal purposes, as milk minimizes the effects of mercuric chloride upon the stomach, without interfering with its systemic action.

Table 4 shows the results obtained when a 10 per cent solution of Merk's serum albumin was used as antidote. It will be noted that the results correspond.

TABLE 4
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) SERUM ALBUMIN (MERCK)

Rabbit	Weight	Mercuric Chloride		Serum Albumin	Lived	Necropsy Lesions	
						Local	General
G1	1000	0.067	w	6.70	3 days	0	Marked
G2	1958	0.078	w	7.00	4 "	0	"
G3	1559	0.062	w	3.10	9 "	0	Slight
G4	1319	0.053	w	4.00	5 "	Slight	Marked

This table shows that Serum Albumin is of but little value as a general antidote to mercuric chloride; animals living an average of 5.25 days after the poisoning.

on the whole, with those obtained with milk, excepting that the average lethal period was a little longer, $5\frac{1}{4}$ days. This was mainly due to the fact that the animal receiving the smallest amount of antidotal solution (G 3, 50 times) lived the longest, while the animal that received the largest quantity (G 1, 100 times),

had the shortest lethal period. This suggests that the warning against giving an excess of albumin in this condition, may be well founded. Still, the results did not seem sufficiently promising to pursue this question any further for the present.

Turning now to the use of egg albumen as antidote (Table 5), we find that the majority of the animals survived, when the albumen was given mixed with the poison. This simply means that mercuric egg albuminate is much less toxic than are the other albuminates studied. Next, egg albumen was administered immediately after the poison was given, and without removing the stomach tube. The result is shown in the second part of Table 5. The average fatal period was 19.2 days, due chiefly to the survival of one animal. That an antidotal effect had been obtained in this case can hardly be postulated, it might be an accidental result. Taking the next step, and administering the antidote 5, 10, 15

TABLE 5
MERCURIC CHLORIDE AND EGG ALBUMEN
Lethal Dose of Mercuric Chloride mixed with (w) Egg Albumen.

Rabbit	Weight	Mercuric Chloride		Egg Albumen	Lived	Necropsy Lesions	
						Local	General
G 5	1775	0.071	w	35.50	81 days	0	0?
G10	1871	0.075	w	37.50	4 "	0	Slight
G11	1235	0.050	w	25.00	43 "	0	Marked
G12	1648	0.066	w	33.00	100 "		
G13	1117	0.045	w	22.50	100 "	0	Pneumonia

Lethal Dose of Mercuric Chloride followed by (f) Egg Albumen.

GL10	2273	0.090	f	45.00	4 days	Slight	Marked Pneu
GL11	1560	0.062	f	31.00	7 "	Marked	Marked
GL12	1885	0.075	f	37.50	3 "	Slight	"
GL13	1055	0.042	f	21.00	80 "	0	?
GL14	1715	0.069	f	35.50	2 "	Marked	Marked

Mercuric Chlor. followed by (f) Egg Albumen after varying intervals.

G 5L	1940	0.078	5 min.	39.00	7 days	Marked	Marked
G10L	1850	0.074	10 "	37.00	3 "	"	"
G15L	2225	0.089	15 "	45.50	1 "	"	"

This table shows that egg albumen is capable of saving life when given mixed with mercuric chloride, but that its antidotal value is rather slight when given immediately afterward, and practically nil when given after an interval of 5 to 15 minutes.

minutes after the poison was given, having in the meantime withdrawn the stomach tube, we find practically no antidotal effect. Inasmuch as these are the conditions that prevail in practise, we must conclude that egg albumen is not likely to be of any value as antidote in the treatment of mercuric chloride poisoning. Evidently the combination that the mercuric chloride forms with the organic materials in the stomach cannot be broken up by albumen.

POTASSIUM IODIDE AND QUININE.

Hall¹ advanced the apparently ingenious suggestion of reversing Mayer's Reagent for the precipitation of mercury, giving the following formula:

Potassium iodide	3.00
Quinine hydrochloride	1.60
Hydrochloric acid, dilute. (10%)	2.00
Distilled water to	100.0

A solution of this composition will remove all the mercury from an equal volume of one per cent mercuric chloride solution, producing a yellow precipitate insoluble in dilute acid and in 0.2 per cent alkali carbonate.

Barbour² tested Hall's alleged antidote by giving it intravenously or intra-peritoneally one-half to one hour after parenteral administration of the poison, with the hope of fixing the mercury, that had already reached the blood and tissues; but with negative results.

That Hall's solution is not antidotal to mercury, even when administered by stomach, I have shown by the following experiments:

TABLE 6
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) POTASSIUM IODIDE AND QUININE (HALL'S SOLUTION)

Rabbit	Weight	Mercuric Chloride		Hall's Sol.	Lived	Necropsy Lesions	
						Local	General
B	670	0.04	w	4 mls	3 days	0	Marked
B 3	610	0.04	w	4 "	2 1/2 "	0	"
B 2	470	0.03	w	6 "	3 "		
BL	650	0.04	f	4 "	3 "	Marked	Marked
BL1	600	0.04	f. 5 min.	4 "	2 "	"	"

Hall's Solution is of no value as antidote to mercuric chloride. Average lethal period 2.7 days.

It will be seen from this table that death occurred even when the supposed antidote was given mixed with the poison (Experiments B and B3), even if used in more than sufficient amount (B2). Necropsy of animals showed kidneys with light cortical striæ. and cecal folds necrotic at their edges. Evidently the mercuric compound is broken up in the digestive tract with liberation of the mercury in active form.

When the solution was given mixed with the poison, there was, of course, no local action; but, if given afterward (B1, and B11), corrosion of the stomach was present.

Hall's solution is therefore useless as an antidote.

TABLE 7
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) SODIUM CARBONATE

Rabbit	Weight	Mercuric Chloride		Sodium Carbonate	Lived	Necropsy Lesions	
						Local	General
D10	860	0.0575	w	0.25	1 day	0	Marked
D11	2235	0.089	w	0.42	3 days	0	"
D12	2110	0.085	w	0.85	4 "	0	"

This table shows that Sodium Carbonate is of no value as a general antidote to mercuric chloride; animals living only an average of 2.7 days after the poisoning.

ALKALIES.

Alkali, in the form of sodium bicarbonate, has been suggested as an antidote by J. M. Hirsch.³ In the *Journal of the American Medical Association* for March 7, 1914, is reported an analysis of an alleged antidote to corrosive sublimate, which is marketed in the form of pink tablets, that on examination

was found to consist of 91 per cent sodium bicarbonate. It, therefore, seemed desirable to study the antidotal value of this substance.

On mixing a mercuric chloride solution with a small amount of sodium bicarbonate, a brown precipitate is formed. If, however, an excess is added, e.g., 25 parts to 1 part of mercuric chloride, there is no precipitation whatever. Inasmuch as sodium carbonate seemed to be superior as a precipitate of mercuric chloride, forming with it a brown precipitant of oxychlorides of mercury, it was first subjected to trial, with the results shown in Table 7. Evidently it has no antidotal value.

Turning now to the results obtained with sodium bicarbonate (Table 8), we note that a certain, though slight, antidotal effect can be obtained from it, one which compares favorably with that obtained from egg albumen.

TABLE 8
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM BICARBONATE

Rabbit	Weight	Mercuric Chloride		Sodium Bicarb.	Lived	Necropsy Lesions	
						Local	General
D 20	1880	0.075	w	0.75	3 days	Marked	Marked
D 21	2330	0.093	w	0.93	9 "	Slight	"
D 23	1415	0.057	w	1.425	100 "	0	Slight
D 24	1042	0.042	w	1.050	3 "	0	Marked
DL21	1010	0.040	f	1.00	14 "	0	"
DL22	1860	0.074	f	1.86	39 "	0	Pneumonia
DL23	1485	0.059	f	1.48	7 "	Slight	Slight
DL24	1315	0.053	f	1.325	8 "	"	Marked

This table shows that Sodium Bicarbonate has antidotal value in mercuric chloride poisoning even when given immediately afterward.
Given with sodium bicarbonate, average survival 29 days.
Followed by sodium bicarbonate, average survival 17 days.

It is an interesting question why sodium bicarbonate, in quantity that does not precipitate mercuric chloride, should be antidotal; while sodium carbonate, which precipitates it, is not. It is probably due to the influence of sodium bicarbonate upon the reaction between mercuric chloride and proteid. When we mix mercuric chloride solution with a serum albumen solution (1:1,000) we obtain a light flocculent precipitate. If, however, the mercuric chloride has previously been mixed with sodium bicarbonate a much denser precipitate is formed. When a solution of sodium bicarbonate is added to a suspension of the flocculent serum albuminate of mercury, we obtain prompt deflocculation of the albuminate.

It will be noted from the reports of the necropsies, that local lesions were slight or absent in all cases, excepting D20, in which case the amount of sodium bicarbonate used was small—only ten times as much as mercuric chloride—while in all others (excepting D21), it was 25 to 50 times as much. We may conclude, therefore, that an excess of sodium bicarbonate renders mercuric chloride non-corrosive when given mixed with it, and that it may possibly have a favorable effect upon the course of the poisoning, even when given afterward.

SODIUM ACETATE.

In view of the fact that there is probably no other metal, the degree of electrolytic dissociation of which, in aqueous solution, is dependent to so high a degree upon the anion with which it is associated, as are the mercuric salts; and, inasmuch as mercuric salts of very weak acids are very much less dissociated than are the salts of stronger acids, it was thought advisable to determine the antidotal value of some of the organic acid anions, for instance, acetate. When we mix mercuric chloride solution with a small amount of sodium acetate, no evident change occurs. Probably mercuric acetate is formed; but, inasmuch as that is soluble and colorless, its production does not manifest itself. When a large excess of sodium acetate is used, we obtain a brown precipitate, identical in appearance with that produced by sodium carbonate and probably composed like the latter of oxychlorides of mercury. This brown precipitate is evidently due to alkalinity. For, if we neutralize the acetate, no such precipitate is ob-

TABLE 9
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F)
SODIUM ACETATE

Rabbit	Weight	Mercuric Chloride		Sodium Acetate	Lived	Necropsy Lesions	
						Local	General
D 30	1680	0.067	w	6.70	8 days	0	Marked
D 31	1040	0.042	w	4.20	100 "	0	Slight
D 32	1700	0.068	w	6.80	3 "	0	"
D 33	1495	0.060	w	6.00	100 "		
D 34	796	0.032	w	3.20	4 "	Slight	Marked
DL30	1650	0.066	f	6.60	12 "	"	Slight
DL31	1865	0.075	f	7.50	100 "		
DL32	1635	0.065	f	6.50	1 "	Marked	Marked
DL33	1793	0.072	f	7.20	5 "	Slight	"
DL34	968	0.039	f	3.90	8 "	"	"

This table shows that a large excess of sodium acetate has considerable antidotal value in mercuric chloride poisoning. Given with sodium acetate, average survival 45 days. Followed by sodium acetate, average survival 25.2 days.

tained. Having determined that it required 100 times the amount of acetate to completely remove the mercuric ions from solution, that amount was given with the result shown in Table 9. An antidotal effect was obtained, ranking somewhat higher than that of sodium bicarbonate or of egg albumen.

POTASSIUM BITARTRATE.

Lambert and Patterson⁴ have recently reported on a method of treatment of corrosive sublimate poisoning, by means of which they scored 100 per cent recoveries in the 10 cases they treated. The treatment consists of a powerful appeal to the emunctories, a veritable washing out of the system, being composed of: (1), the administration of eight ounces of potassium bitartrate lactose lemonade every two hours and of eight ounces of milk every two hours, each being given alternate hours, and gastric lavage twice daily in addition; (2), the continuous drop rectal irrigation with a solution of potassium acetate, and copious enteroclysis twice daily, besides; (3), a sweat bath daily. The antidotal treatment their cases received consisted of the administration of albumen which,

as we have shown, is probably of little importance in producing the results they obtained, as it was always given some time after the poison had been taken. To determine whether the potassium bitartrate, which their patients received, had any antidotal value, I performed experiments, the results of which are shown in Table 10. It will be seen that potassium bitartrate has practically no antidotal value. We may, therefore, admit from their results, that heroic eliminant treatment may be of value in mercurial poisoning, even if an inefficient antidote is used. Of course, a combination of such treatment with the use of a good antidote, ought to be still better.

TABLE 10

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) POTASSIUM BITARTRATE
(25 TO 50 TIMES AS MUCH)

Rabbit	Weight	Mercuric Chloride		Potassium Bitartrate	Water	Lived	Necropsy Lesions	
							Local	General
T 1	1540	0.061	w	3.50	35.00	5 days	Slight	Marked
T 2	1815	0.073	w	1.82	18.00	6 "	"	"
T 3	1483	0.059	w	3.00	30.00	4 "	Marked	"

Potassium Bitartrate has a very slight antidotal value. Animals living an average of 5 days after poisoning.

SODIUM SULPHATE.

In view of the report of Macht,⁵ that sodium sulphate was of value in phenol poisoning, and assuming that the fact that sodium sulphate is an excellent deflocculent for proteids, might explain the action, I performed test tube experiments to determine whether this was true for mercuric serum albuminate sol. Having found that this was the case, I tried it on a few poisoned rabbits, as shown in Table 11, with perfectly negative rabbits.

TABLE 11

LETHAL DOSE OF MERCURIC CHLORIDE FOLLOWED BY (F) SODIUM SULPHATE
(50 TO 100 TIMES AS MUCH)

Rabbit	Weight	Mercuric Chloride		Sodium Sulphate	Water	Lived	Necropsy Lesions	
							Local	General
SL1	1530	0.061	f	6.10	24.4	5 days	Slight	Marked
SL2	1147	0.046	f	4.60	25.6	1 day	"	Slight
SL3	1355	0.054	f	2.70	17.4	2 days	Marked	Marked

Sodium Sulphate has no antidotal value. Animals living only 2²/₃ days average.

STANNOUS CHLORIDE.

As is well known, there is a great difference in the toxicity of mercuric salts and that of mercurous salts and of mercury. When, therefore, we reduce mercuric chloride to a mercurous salt, or to a metallic mercury, an antidotal effect ought to be obtained. On comparing a series of reducing agents, I found stannous chloride the most active. An equal part of one per cent solution of stannous chloride, added to a one per cent solution of metallic mercury, produces a grey precipitate and removes practically all mercuric ions from the solution.

Having determined that the lethal dose of stannous chloride for the rabbit is about 1 gm. per kg. (Table 12), I administered it in safe doses mixed with the corrosive sublimate. (See Table 13.)

TABLE 12
DETERMINATION OF LETHAL DOSE OF STANNOUS CHLORIDE

Dose per kg. Rabbit	Number of Experiments	Result
0,25	1	Recovered
0,50	1	"
1,00	2	1 Died
2,00	1	"

Showing that lethal dose is about 1,00 gm. per kg.

TABLE 13
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F)
STANNOUS CHLORIDE

Rabbit	Weight	Mercuric Chloride		Stannous Chloride	Lived	Necropsy Lesions	
						Local	General
F	620	0.040	w	0.080	50 days	0	Marked
F 1	1195	0.048	w	0.096	16 "	0	"
F 2	960	0.039	w	0.077	7 "	0	"
F 3	823	0.033	w	0.210	100 "	0	Pneumonia
F 4	1205	0.048	w	0.480	16 "	0	"
FL	890	0.060	f	0.120	3 "	Marked	Marked
FL 1	1055	0.042	f	0.084	2 "	"	"
FL 2	1095	0.044	f	0.088	2 "	Slight	"
FL 3	845	0.034	f	0.210	17 "	Marked	"
FL 4	1370	0.055	f	0.550	8 "	Slight	Pneumonia

This table shows that stannous chloride is a good antidote when mixed with the poison; but of much less value when given afterward.
Given with stannous chloride, average survival 37 days.
Followed by stannous chloride, average survival 6 days.

A decided antidotal action, a lengthening of the average fatal period to 37 days, was obtained. When, however, the stannous chloride was administered immediately after the poison, the result was disappointing. The average fatal period was merely six days. The reason for this discrepancy between the fatal period under these two conditions of administration of the antidote, is probably due to the chemical instability of the stannous chloride, and the fact that it is a proteid precipitant. Hence, if administered afterward, it will react with other substances quite as readily as with mercuric chloride. Perhaps, if larger doses were used, it might give better results. The best results, shown in Table 13, have been obtained with the largest doses. Still, as we have less toxic and more useful antidotes among the reducing agents, no further studies were made with stannous chloride.

CALCIUM SULPHIDE.

Hayward and Allen,⁶ in a letter to the editor of the *Journal of the American Medical Association*, suggested the use of calcium sulphide as an antidote to mercuric chloride poisoning, on the ground that 1 gm. of calcium sulphide (calx

sulphurata, U. S. P.) is capable of reducing 2.31 gm. of mercuric chloride. They report no experimental work upon animals.

To determine the toxicity of the proposed antidote, I administered to a rabbit weighing 1,276 gm. a dose of 0.10 gm. of calcium sulphide per kg., by means of a stomach tube. Within three minutes the animal was lying on its side, panting, its breath smelling of hydrogen sulphide, pupils dilated, corneal reflex sluggish. A convulsion occurred within ten minutes after the administration. The animal remained lying on its side, its ears becoming bluish; and it seemed to be dying. It commenced to improve, however, within twenty-five minutes, though it remained depressed, did not eat, and died on the fourth day after the administration of the agent; necropsy showing multiple punctate hemorrhages in the stomach.

It appears that an agent as highly and rapidly toxic as calcium sulphide, would not be likely to be of value as an antidote, especially in view of the fact

TABLE 14
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F)
SODIUM PHOSPHITE

Rabbit	Weight	Mercuric Chloride		Sodium Phosphite	Lived	Necropsy Lesions	
						Local	General
H	1018	0.068	w	0.68	4 days	0	Marked
H 1	780	0.030	w	0.30	4 "	0	"
HL1	1188	0.047	f	0.47	4 "	Marked	"

Sodium Phosphite given alone is of little value as antidote, av. survival 4 days.

that we have other reducing agents that are much less toxic. I therefore did not carry the experiments with this agent any further.

SODIUM PHOSPHITE.

Thomas A. Carter⁷ announced, in 1914, a series of 16 cases of mercuric chloride poisoning, treated with a new antidote, with only two deaths. He has since enlarged his series to 74 cases with only four deaths.⁸ His antidote is composed of sodium phosphite, six parts, and sodium acetate, four parts.

When a solution of sodium phosphite is mixed with a mercuric chloride solution, a white precipitate (calomel) is formed; and if the proportions are correct—ten parts of sodium phosphite to one part of mercuric chloride—practically all of the mercury is removed from the solution.

I first administered the sodium phosphite mixed with and following a fatal dose of mercuric chloride, without obtaining any antidotal effect (Table 14); then mixed it with a liberal excess of sodium acetate, which had previously been found to have an antidotal value; but found that the combination was devoid of antidotal action.

Experimenting then with the proportions given by Carter, using 10 parts of sodium phosphite to 6.6 parts of sodium acetate, I obtained the results shown in Table 16, an evidently antidotal effect even if the antidote was given after the poison was administered. The average fatal period was lengthened to 61.6 days, when the poison and antidote were given combined; and to 42.4 days, when the

antidote was given afterward. Why the combination in the proportions stated should be so much more effective than the same combination with an excess of acetate, which in itself has antidotal value, I am unable to explain.

SODIUM HYPOPHOSPHITE.

Theoretically, sodium hypophosphite should be a better reducing agent than sodium phosphite, for it represents a still lower state of oxidation. However, it seems to be somewhat slower than the phosphite in undergoing reaction with mercuric chloride, though the result is the same, a white precipitate of calomel

TABLE 15
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM PHOSPHITE (10 TIMES AS MUCH) AND SODIUM ACETATE (100 TIMES AS MUCH)

Rabbit	Weight	Mercuric Chloride		Sodium Phosphite	Sodium Acetate	Lived	Necropsy Lesions	
							Local	General
H 4	1749	0.070	w	0.70	7.00	1 day	Slight	Slight
H 6	974	0.039	w	0.39	3.90	3 days	"	"
HL4	1215	0.049	f	0.49	4.90	3 "	Marked	Marked
HL6	1000	0.040	f	0.40	4.00	4 "	Slight	"

Sod. Phosphite with large excess of acetate is of no antidotal value.

TABLE 16
LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM PHOSPHITE (TEN TIMES AS MUCH) AND SODIUM ACETATE (6.6 TIMES AS MUCH)
(CARTER'S ANTIDOTE)

Rabbit	Weight	HgCL ₂		Sodium Phosphite	Sodium Acetate	Lived	Necropsy Lesions	
							Local	General
H 2	1665	0.067	w	0.67	0.442	1 day	0	Slight
H 3	1870	0.075	w	0.75	0.50	100 days	Slight	Ear Inf.
H 5	1650	0.066	w	0.66	0.44	100 "		
H 7	1525	0.061	w	0.61	0.40	7 "	Marked	Marked
H 8	1550	0.062	w	0.62	0.41	100 "		
HL2	1795	0.072	f	0.720	0.475	100 "	0	Slight
HL3	2075	0.113	f	1.13	0.75	4 "	0	Pneum.
HL5	1877	0.075	f	0.75	0.50	100 "		
HL7	2100	0.084	f	0.84	0.554	6 "	Marked	Marked
HL8	1440	0.058	f	0.58	0.38	2 "	Slight	"

Carter's Antidote is of decided value.
Average survival when given with the poison 61.6 days.
Average survival when given following the poison 42.4 days.

being formed. Finding in an experiment, in which I administered it mixed with the poison, that the fatal period was merely prolonged to eight days, I proceeded to use the sodium hypophosphite in various combinations, having noticed that combination with a third agent seemed to hasten the speed of the reaction.

Combination of sodium hypophosphite with sodium acetate in proportion of 10 to 6.6 gave the results shown in Table 17.

While the figures representing the number of days the animals survived, give a somewhat lower average than was obtained with Carter's Antidote, 37.6, as compared with 42.4, still a comparison of Tables 16 and 17 will show that

the results were somewhat more uniform with the hypophosphite, not one animal among those that were given the hypophosphite dying in less than 20 days, while with Carter's antidote, one animal died in one day and one in seven days. Furthermore, most of the animals of the hypophosphite series succumbed to rabbit pneumonia and similar infections, which had unfortunately attacked them in an epidemic manner. Taking, however, the figures as they are, in spite of this handicap, we find them to rank the hypophosphite and acetate well alongside of the phosphite combination.

TABLE 17

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM HYPOPHOSPHITE (10 TIMES AS MUCH) AND SODIUM ACETATE (6.6 TIMES AS MUCH)

Rabbit	Weight	HgCl ₂		Sodium Hypo.	Sodium Acetate	Lived	Necropsy Lesions	
							Local	General
Ca 2	1205	0.048	w	0.48	0.32	60 days	0	Nose Inf.
Ca 4	1392	0.056	w	0.56	0.37	28 "	0	Ear "
Ca 5	965	0.039	w	0.39	0.238	100 "		
Ca 6	1395	0.056	w	0.56	0.37	20 "	0	Pneum.
Ca 7	960	0.038	w	0.38	0.25	38 "	0	"
Cal 2	1437	0.057	f	0.57	0.38	7 "	Slight	Marked
Cal 4	1000	0.040	f	0.40	0.264	67 "	"	Abscess
Cal 5	2450	0.098	f	0.98	0.669	9 "	"	Marked
Cal 6	1115	0.045	f	0.45	0.30	100 "		
Cal 7	1060	0.042	f	0.42	0.28	5 "	0	Slight

Sodium Hypophosphite with sodium acetate has decided antidotal value.

Average survival when given with the poison, 49.2 days.

Average survival when given following the poison, 37.6 days.

TABLE 18

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM HYPOPHOSPHITE (10 TIMES AS MUCH) AND SODIUM ACETATE (100 TIMES AS MUCH)

Rabbit	Weight	Mercuric Chloride		Sodium Hypo.	Sodium Acetate	Lived	Necropsy Lesions	
							Local	General
Ca 1	1100	0.044	w	0.44	4.40	2 days	Slight	?
Ca 3	1128	0.045	w	0.45	4.50	1 "	"	Slight
Cal 1	1335	0.053	f	0.53	5.30	100 "		
Cal 3	965	0.039	f	0.39	3.90	7 "	Slight	Marked

Hypophosphite is of much less antidotal value when given with excess of acetate. Average survival being 27.5 days.

An excess of sodium acetate, added to the hypophosphite, Table 18, lowers the average lethal period as it did in case of phosphite; but not to the same extent. An average survival of 27.5 days was obtained, as against three days in case of phosphite.

Acidified solution of sodium hypophosphite (Table 19), as well as alkalinized solution of this salt (Table 20) were also tried, with results that were not sufficiently encouraging to demand further study.

The best results of all were obtained with sodium hypophosphite to which a moderate amount of hydrogen peroxide was added (5 times as much). The average lethal period (Table 21), when this antidote was administered immediately after the poison, was 43.4 days. When, however, an excess of hydrogen

TABLE 19

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM HYPOPHOSPHITE ACIDIFIED WITH DILUTE HYDROCHLORIC ACID

Rabbit	Weight	Mercuric Chloride		Hypophosphite	Lived	Necropsy Lesions	
						Local	General
C	460	0.030	w	0.30	35 days	Slight	Marked
C 1	770	0.030	w	0.30	1.5 "	Marked	"
C 2	530	0.040	w	0.20	10 "	Slight	"
CL 1	1107	0.044	f	0.44	19 "	0	"
CL 2	1070	0.043	f	0.43	8 "	Slight	"
C 20	547	0.035	f	0.35	7 "	"	"
C 21	860	0.057	f	0.57	5 "	Marked	"

Average lethal period when given with the poison, 15.5 days.
Average lethal period when given following the poison, 9.75 days.

TABLE 20

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM HYPOPHOSPHITE AND SODIUM CARBONATE

Rabbit	Weight	Mercuric Chloride		Hypophosphite	n/5 Na ₂ CO ₃	Lived	Necropsy Lesions	
							Local	General
C 3	1555	0.060	w	0.60	6.00	36 days	Slight	Abscess
C 5	1070	0.043	w	0.43	4.30	28 "	0	Slight
CL 5	915	0.037	f	0.37	3.70	7 "	Marked	Marked
C 30	612	0.040	f	0.40	4.00	6 "	Slight	Marked

Average lethal period when given with poison, 32 days.
Average lethal period when given following poison, 6.5 days.

TABLE 21

LETHAL DOSE OF MERCURIC CHLORIDE FOLLOWED BY (F) SODIUM HYPOPHOSPHITE (10 TIMES AS MUCH) WITH HYDROGEN PEROXIDE (50 TIMES AS MUCH)

Rabbit	Weight	HgCl ₂		NaPH ₂ O ₂	H ₂ O ₂	Lived	Necropsy Lesions	
							Local	General
CHL 4	1150	0.046	f	0.46	2.00	8 days	0	Pneum.
CHL 1	1470	0.059	f	0.59	3.00	60 "	0	?
CHL 2	1645	0.066	f	0.66	3.00	45 "	0	?
CHL 5	2390	0.096	f	0.96	4.80	100 "		
CHL 6	2270	0.091	f	0.91	4.50	4 "	0	Marked

Sodium Hypophosphite with hydrogen peroxide has considerable antidotal value.
Average lethal period, 43.4 days.

TABLE 22

LETHAL DOSE OF MERCURIC CHLORIDE FOLLOWED IMMEDIATELY BY (F) SODIUM HYPOPHOSPHITE (10 TIMES AS MUCH) WITH HYDROGEN PEROXIDE (100 TIMES AS MUCH)

Rabbit	Weight	HgCl ₂		NaPH ₂ O ₂	H ₂ O ₂	Lived	Necropsy Lesions	
							Local	General
CHL 3	1942	0.078	f	0.78	7.80	11 days	0	Marked
CHL 4	1737	0.070	f	0.70	7.00	4 "	Slight	"

This table shows that an excess of hydrogen peroxide is not as good as when a smaller amount is used. Average lethal period being 7.5 days.

peroxide was given with the hypophosphite, the results were much less favorable (Table 22). Whether a still smaller proportion of hydrogen peroxide would give still better results, remains to be determined. Why hydrogen peroxide, an oxidizing agent, should increase the efficiency of a reducing agent, like hypophosphite, is an interesting question. It may be it acts as a catalyzer.

The results of special interest obtained with various antidotes administered after the poison was given, may be most readily compared by reference to accompanying diagram. This shows, at a glance, that sodium phosphite and sodium hypophosphite, in the combinations mentioned, gave the best results.

SUMMARY OF AVERAGE LETHAL PERIODS.

Mercuric Chloride given alone.	
Mercuric Chloride followed by Stannous Chloride.....	
Mercuric Chloride followed by Sodium Bicarbonate.....	
Mercuric Chloride followed by Egg Albumin.....	
Mercuric Chloride followed by Sodium Acetate.....	
Mercuric Chloride followed by Sod. Hypophosphite with Acetate	
Mercuric Chloride followed by Sod. Phosphite with Acetate.	
Mercuric Chloride followed by Sod. Hypophosphite with Hydrogen Peroxide.....	

To determine next whether these antidotes are likely to be of use in the conditions met with in actual practice, each of these three antidotes was administered, 5, 10 and 15 minutes after the poison was given, the stomach tube having been withdrawn meanwhile. In all cases distinct antidotal effects were obtained. One rabbit each, was used in these experiments. The animals that received hypophosphite and acetate 5, 10 and 15 minutes afterward lived, 29, 65 and 9 days respectively. Those that were given hypophosphite and hydrogen peroxide 5, 10 and 15 minutes afterward, lived 15, 28 and 60 days respectively, while of the animals that were given Carter's antidote 5, 10 and 15 minutes afterward, died after 65, 74 and 100 days respectively. Inasmuch, as quite a number of these animals died of rabbit pneumonia, and as only one animal was used for each of these tests, the results do not permit conclusions as to the relative merits of these antidotes. They do, however, prove conclusively that antidotal effects can be obtained from all of them, when they are administered some time after the poison. This, as we have seen (Cf. Table 5) was not the case with egg albumen, as that proved quite worthless when given 10 and 15 minutes after the poison.

CONCLUSIONS.

1. Egg albumen is of little value as an antidote to mercuric chloride, unless it is given immediately after the poison is swallowed. Milk and serum albumin are worthless.
2. Hall's solution (potassium iodide and quinine), is useless as an antidote.

3. Sodium bicarbonate and sodium acetate possibly have a moderate antidotal value. Sodium carbonate is of no value.

4. Potassium bitartrate and sodium sulphate have no antidotal value.

5. Stannous chloride has little antidotal value.

6. Calcium sulphite is probably too toxic to be of use as an antidote.

7. Sodium phosphite alone has no antidotal value; but mixed in a certain proportion with sodium acetate (Carter's antidote) it is of remarkable antidotal efficiency.

8. Sodium hypophosphite mixed with a certain proportion of sodium acetate or of hydrogen peroxide is also highly efficacious as an antidote.

TREATMENT RECOMMENDED.

As the result of my studies, I would recommend the following antidotal treatment for mercuric chloride poisoning: Immediate administration of antidote, either Carter's antidote, a tablet composed of sodium phosphite, 0.36 gm. and sodium acetate 0.24 gm. If this be not available, I would give the following

Sodium Hypophosphite	1.00 gm.
Water	10.00 mils.
Hydrogen Peroxide	5.00 mils.

If the amount of the poison taken be known, ten times as much of the hypophosphite should be given as poison was taken. As this might require a large and possibly harmful amount of hypophosphite, it should immediately be followed by copious lavage, with a very dilute solution of the antidote. This may be followed by a safe dose of the antidote, which is to be retained, and which might be repeated every eight hours for several days.

This antidotal treatment might be combined with some such eliminant treatment as that recommended by Lambert and Patterson,⁴ excepting that sodium acetate might be preferred to the potassium bitartrate given by stomach, because of its superior antidotal value.

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BENCE-JONES PROTEIN*

A PRELIMINARY REPORT.

BY W. A. GROAT, M.D., AND R. K. BREWER, M.D.,
SYRACUSE, NEW YORK.

THE subject of the study of which this is the preliminary report is a man 37 years old, referred to one of us by Dr. W. H. Kidder, of Oswego, N. Y., because of a peculiar albumin reaction found in his urine. This albumin was found by one of us to be Bence-Jones protein, and arrangements were made whereby the patient voluntarily put himself under full control in hospital and study of his case and condition was undertaken and is under way.

Reference to literature has revealed to us no case in which definite association with kidney change has been proved. In fact, the observations are, in the main, that serum albumin proper is not present. However, Hopkins and Savory¹ although stating that in none of their three cases was there evidence of a nephritis, did find, in the only one of these which went to autopsy, definite post-mortem evidence of interstitial kidney change. Folin and Denis² note the regular appearance of a few hyaline and granular casts, but they also state there was no clinical evidence of nephritis. Taylor and Miller in a recent article³ report complete absence of serum albumin in their case. Their method of testing, however, we believe may be open to question, so far as proof of absence of serum albumin is concerned. Recognizing the fact that traces of serum albumin are not easily proved up in otherwise normal urine, we cannot regard complete clearing of the urine when boiled with nitric acid, as they suggest, as proof of the absence of small amounts of true serum albumin, since the resolution of traces of serum albumin in hot nitric acid is accepted fact. Furthermore, using the method of Hopkins and Savory¹, heating with acetic acid for two hours at 60° and cooling on ice to give an albumin free filtrate, we find that in our case all the Bence-Jones albumin is not thereby removed. With these observations in mind, and with the facts as regards the finding and proving of traces of serum albumin and the differentiation of questionable reactions from those of so-called nucleo-albumin, et cetera, under like conditions, we form the opinion that the presence or absence of true serum albumin and kidney change have not yet been proved. A reference to the complete work of Hopkins and Savory¹, showing the varying behavior of Bence-Jones protein in their three cases with graded amounts of salt and acid, clearly shows how Bence-Jones protein may complicate the matter of proof of the presence or absence of serum albumin. We, therefore, have approached the matter from a new angle; and briefly, we find in our case an excretion of but 20 per cent of phenolsulphon-phthalin, with a blood pressure of 130 to 135, in a man anemic and much depleted. Typical urine analyses with complete nitrogen partition, together with the blood analysis showing nitrogen retention, are tabulated below.

*From the Hazard Memorial Laboratory, Syracuse Hospital for Women and Children, and the Chemical Laboratory, College of Medicine, Syracuse University.

BLOOD ANALYSIS.

Non Protein N=	81.9	
Urea N	40.8	Creatinine 2.1
Uric Acid	6.2	Creatine+creatinine 11.5

URINE ANALYSES.

I.		II.
17.56	Total N.	16.55
12.42	Non Protein N.	10.20
5.14	Protein N.	6.35
32.38	Bence-Jones Protein	38.17
10.38	Urea N.	7.45
.31	Ammonia N.	.27
.23	Uric Acid N.	.30
.67	Creatinine N.	.63
None	Creatine	None

Briefly stated, our case seems to be a quite typical one clinically as regards general symptomatology. The urine shows a few hyaline and granular casts as has been noted in previous cases, but no ordinary clinical evidence of a nephritis. The quantity of the protein has varied from 32.4 to 41.3 grams in 24 hours, irrespective of diet.

It is not difficult to understand how the excretion of such an amount of an abnormal urinary constituent could cause true kidney change. Our case appearing to be a typical one and taking particular note of the interstitial kidney change found in the one case at autopsy,¹ and finding of hyaline and granular casts in other cases, notably Folin and Denis,² our findings of a nitrogen retention, while standing alone as far as we can find from the search of literature, are, nevertheless, also the only instance we find in which such determinations have been reported, and taken altogether, are, we believe, of particular interest. A full clinical report and a full report of laboratory study now under way are to appear. This is but a preliminary report.

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DETECTION OF SMALL AMOUNTS OF BLOOD

BY T. H. KELLY, M.D., CINCINNATI, OHIO.

THERE have been many attempts made to devise methods which would accurately indicate the presence or absence of blood in fluids, stains, etc., but as yet no chemical method, with one exception, has been evolved which will enable us, without confirmatory examinations, to say definitely that we are dealing with blood, whatever its origin. The exception is the hemin crystal test of Teichmann, which is specific for hemoglobin; this is not delicate enough to detect such minute quantities of blood as we have to deal with in certain pathologic urines, stools, etc.

The diagnostic importance of the presence of blood in certain of the human excretions or secretions, is certainly not less than its significance in stains, etc. in forensic medicine, and so the knowledge of the value and technic of the various tests becomes a necessity to every man in medicine.

The most widely used test in all probability is the guaiac test, which was introduced first by Schonbein¹ in 1856. This test depends, as do all others which have been extensively used, upon the fact that hemoglobin, or its iron containing derivatives, in the presence of oxygen acts as an oxygen carrier, thereby oxidizing certain colorless compounds into one showing distinct color. The other substances which have been used in this type of test are aloin, benzidine, the leuco-base of malachite green and phenolphthalin.

There has been, and still is, considerable question as to the exact method by which the blood is able to bring about the oxidation of these chromogenic substances in the presence of oxygen. Delearde and A. Benoit² think that the blood acts as a peroxidase. Kastle and Amoss³ have called it the "peroxidase activity" of the blood, citing the facts that blood differs in its reactions from peroxidases in that it does not lose the oxygen carrying activity after boiling, and that it reacts in such concentrations of alkali as would destroy most, if not all of the true peroxidases. Further, the activity was found to vary with the hemoglobin content. It has also been shown that the oxygen carrying activity of the blood persists after boiling and treatment with acids and alkalies, until the blood pigments are deprived of their iron (Mortessier⁴, Czyhlarz and von Furth⁵, Lesser⁶, Whitney⁷). Kastle⁸ gives an hypothesis which supposes that the iron-containing blood pigment combines with the peroxide of hydrogen to form a compound which gives up its oxygen to the chromogenic substance more easily than the peroxide itself, resulting in the production of a dye of a given color.

There have been objections urged against these tests because of the fact that certain substances other than blood have been shown to give the guaiac or similar reactions. Kastle⁸, in his monograph, gives a list, occupying three pages, of substances which have been found to give these reactions, the most important clinically being pus, milk, saliva, bile and feces (after meals containing raw or cooked meats or foods containing blood). Also of occasional importance clinically are the salts of iron and mercury. Needless to say, in the performance of these tests the clinician must do his best to assure himself that these sources of

error are eliminated as far as possible before the test is attempted. In testing for blood in feces we must be certain that the patient has not been eating meat recently. This can be accomplished by putting him upon a meat free diet, and marking the beginning of this regime by a dose of charcoal, carmine, etc., which will color the feces, thereby marking the commencement of meat free stools.

The technic of the guaiac test is as follows: A part of the material to be tested is thoroughly mixed with one-third of its volume of glacial acetic acid, and then an equal part of ether is added and the mixture well shaken. The ethereal extract thus made is permitted to separate, a few drops of alcohol often materially hastening the separation. The ethereal extract is poured off into a clean test tube and ten drops of a freshly prepared tincture of guaiac (alcoholic solution of gum guaiac 1:5) and 20-30 drops of ozonized (old) turpentine added. In the presence of blood the mixture assumes a violet blue color and in its absence it turns red-brown or greenish. The color may be more definitely seen in doubtful reactions by adding a little chloroform, shaking the mixture well, and allowing the chloroform to settle out. The chloroform extracts the blue pigment, and is distinctly colored by it.

According to Weber⁹ potato and other starches, as well as bile, milk, saliva, pus and preparations of iron produce the blue coloration, and he recommends the acid-ethereal extract as outlined above, saying that this procedure obviates the above errors.

There is a modification of the test which makes it somewhat more convenient, and often renders the reaction more distinct in doubtful cases. Instead of using old turpentine, the tincture of guaiac is freshly prepared and 5 c.c. placed in a test tube. Two or three drops of a 3 per cent solution of hydrogen peroxide are added to this and then a few cubic centimeters of the ethereal extract to be tested is layered above it. If blood is present a blue ring forms at the line of contact within a minute or two, and if blood is absent only a very slight brownish discoloration appears and often none at all. The colored ring is very easily seen if present, there being very little production of other pigments to obscure the color.

Boas¹⁰ says that this test is not decisive in the feces since the blue is often overshadowed by the browns, and he recommends the use of aloin as suggested by Klunge, Schar and Rossel.

This test is performed according to Rossel¹¹ as follows: 5 c.c. of stool are extracted with ether to remove any fats which may be present, as they interfere later by the production of emulsions. After removal of the ether extract 3-5 c.c. of glacial acetic acid are added to the feces, and this mixture is again extracted with ether. This acid-ethereal extract is now used for the test. As much aloin as can be placed upon the tip of a pen-knife is dissolved in 3-5 c.c. of 70% alcohol and 10-15 drops of this solution are added to the acid-ethereal extract after first adding to it 20-30 drops of resinous oil of turpentine, or according to Brandenburg, a dilute solution of hydrogen peroxide. In the presence of blood, the mixture rapidly becomes bright red, later assuming a cherry color. In the absence of blood, it becomes reddish only after an hour or two. According to Boas, the reaction can be hastened by adding a few drops of chloroform.

This test can also be performed as in the modification of the guaiac reaction.

The benzidine reaction is done as follows: As much benzidine as will go on the end of a small knife is placed in 2 c.c. of glacial acetic acid and shaken. A piece of feces the size of a pea is placed in 5-10 c.c. of water and boiled, the test tube being closed by cotton. Then 10-12 drops of the benzidine solution are placed in a test tube and 2-2.5 c.c. of 3% hydrogen peroxide added. 1-5 drops of the boiled feces are added, greenish or blue color denoting presence of blood, and appearing in two minutes if blood is present in dilution of 1-200000. This test is 5-10 times more delicate than the preceding ones.

In 1903 Meyer¹² used phenolphthalin as a reagent for leucocytes. In the same year Utz¹³ used a solution of phenolphthalin in sodium carbonate for the determination of blood in legal investigations. In 1906 Kastle and Amoss³ used an alkaline solution of phenolphthalin to determine the oxidizing power of blood in health and disease. They showed that both its oxidizing power and its peroxidase activity (its power to induce the oxidation of phenolphthalin by hydrogen peroxide in alkaline solution) depended directly upon its hemoglobin content. Delearde and A. Benoit in 1908 found an alkaline solution of phenolphthalin to be more sensitive than guaiac or aloin as a reagent for blood.

Phenolphthalin is the colorless compound of phenolphthalein and on oxidation is converted into phenolphthalein. Pure phenolphthalin is colorless in alkaline solutions, but on adding alkali to it after oxidation, or on oxidizing it in alkaline solution, the deep purplish-red color of phenolphthalein is produced. Considerable difficulty has been experienced by workers in this field in the preparation of the reagent, but Kastle⁸ says that by the following method phenolphthalin can be prepared which is very stabile if kept in glass stoppered bottles in dark closets. The method which he used is given in his own words: "Phenolphthalein is dissolved in a considerable excess of 30% sodium hydroxide solution and boiled with an excess of zinc dust until a few drops of the strongly alkaline liquid no longer give the color of phenolphthalein after neutralization with hydrochloric acid and the addition of sufficient alkali to give a slightly alkaline reaction. The solution is then decanted from the excess of zinc dust and the phenolphthalin precipitated by acidifying with hydrochloric acid. The substance is then collected on a filter and purified by several crystallizations from water and alcohol in the following manner: The phenolphthalin is dissolved in the smallest quantity of boiling alcohol in which it will dissolve, filtered if necessary, and cold water gradually added with constant stirring until the compound is precipitated out as a white crystalline precipitate. From three to five crystallizations are carried out in precisely this manner, and in this way the phenolphthalin is finally obtained in the form of a white crystalline compound free from all traces of phenolphthalein. It may then be dried in the air at ordinary temperature or on the hot plate or in the oven at temperatures ranging from 50° to 80° C. In handling and drying the compound it is necessary to keep it out of contact with all metallic surfaces, and also to prevent access of dust and impurities from the laboratory."

Phenolphthalin can now be purchased in the market, however, thus obviating the necessity of making the substance for use in the clinical laboratory.

The reagent phenolphthalin may be used for the detection of blood in either

one of two ways or both—i. e., as the alkaline phenolphthalin or as the alkaline phenolphthalin-hydrogen peroxide solution.

The alkaline phenolphthalin reagent is prepared as follows according to Kastle:⁸ To 21 c.c. of N/10 sodium hydroxide is added 0.032 gram of phenolphthalin, and then enough distilled water to make 100 c.c. In the absence of a delicate balance Kastle says that the following method may be followed: 1 c.c. of N/10 sodium hydroxide is placed in contact with somewhat more phenolphthalin than will dissolve in it, diluted with 10 to 20 c.c. of water, filtered and then to the filtrate is added 20 c.c. of N/10 sodium hydroxide and enough distilled water to make 100 c.c. This can be done because the phenolphthalin is insoluble in water and 1 c.c. of N/10 sodium hydroxide neutralizes exactly 0.032 gram of the reagent.

The alkaline phenolphthalin-hydrogen peroxide reagent is prepared as follows (Kastle⁸). The procedures are the same as for the former reagent except that in making all the solutions, water must be used which has been redistilled in glass vessels, since occasionally small amounts of copper from the still will produce a positive reaction. After the alkaline solution is made 0.1 c.c. of M/1 hydrogen peroxide is added before diluting to 100 c.c. In ordinary practice it is sufficiently accurate if 0.1 c.c. of the 3% commercial hydrogen peroxide is used. This solution, if kept in the dark in glass stoppered bottles at ordinary temperature, will remain practically colorless for 48 hours.

In clinical practice the alkaline phenolphthalin reagent may be used and a drop of peroxide added at the time of making the tests. The test for blood is made by mixing one part of the material to be tested and two parts of the reagent. After five minutes the alkaline phenolphthalin reagent containing hydrogen peroxide should show color in the presence of very small amounts of blood.

Kastle was able by very careful technic to detect the oxidizing effect of 0.000000038 gram of blood in a total dilution of 3 c.c. or approximately 1 part of blood in 80,000,000 parts of water. He was able to measure colorimetrically the oxidizing power of blood at a dilution of 1 to 8,000,000 parts of water, this taking into consideration the volume of the reagent also. It was also possible to detect 8 parts of blood in 1,000,000 parts of urine.

However, the presence of other organic substances renders the test much less delicate than this, though it still exceeds greatly the sensitiveness of guaiac and benzidine. The retarding effect can be partially removed by utilizing the fact that blood pigments are absorbed by aluminum hydroxide, as first shown by Rose. Three or four drops of thick alumina cream are mixed with the suspected fluid and the mixture shaken well, filtered and the residue washed with a little distilled water. The residue is then used to perform the test.

Thus, in the phenolphthalin reagent we have an extremely delicate test for blood, and while there are many substances which also produce the reaction, their occurrence in the various secretions and excretions which are of interest to the clinician is rather uncommon, at least in quantities large enough to disturb the results. If there is any doubt, it can very often be settled by the behavior of the substance toward both the reagent containing peroxide and the one without it. Blood has the power of oxidizing phenolphthalin either in the presence or

absence of hydrogen peroxide, while the other substances vary in their activity toward one or the other reagents, none of them showing oxidizing power toward alkaline phenolphthalin, with and without hydrogen peroxide, comparable to blood.

J. Boas¹⁴ has recently described an improvement of the phenolphthalin reaction for the demonstration of occult blood in the feces and stomach contents. He uses a phenolphthalin solution prepared by adding 1 gm. of phenolphthalin to 100 c.c. of distilled water in which 25 g. KOH have been dissolved. This solution which is not colorless is then heated with the addition of metallic zinc until it is colorless, this procedure usually requiring 1-2 hours. The solution is cooled, brought up in the original volume and filtered. It will keep for several weeks.

In making tests of feces, a glacial acetic acid-alcohol extract of the feces is made, using a mixture of 5 drops of glacial acetic acid to 15-20 g. of alcohol. Fifteen drops of the phenolphthalin reagent are put in a test tube, 5-6 drops of 3% hydrogen peroxide, and 2 c.c. of absolute alcohol are added, and the mixture shaken. The acetic acid-alcohol extract of the feces is run in slowly along the side of the test tube in order to obtain good stratification, and if blood is present in the feces, a pink to deep red ring is formed at the line of contact. This test performed in this manner is very sensitive for the examination of feces, but if stomach contents are to be examined they must be extracted with a mixture of glacial acetic acid and ether.

Good results may be obtained by testing glacial acetic acid-alcohol extracts of stomach contents with guaiac tincture.

These are the most widely known and reliable tests for occult blood that we have, and, though all of them give positive reactions with substances other than hemoglobin, exclusion of these other substances which are known to give the reactions enables us to say that in the presence of a positive reaction we are dealing with derivatives of blood. This means careful attention to the methods of obtaining specimens for examination, accuracy in the performance of these reactions, and intelligence in the interpretation of results.

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THE TEMPERATURE REACTIONS IN ANAPHYLAXIS*

BY MAURICE I. SMITH, M.D., ANN ARBOR, MICH.

KREHL¹ in 1895 studying the temperature effects of different proteins introduced parenterally in animals noticed that "used animals" responded with a greater rise in temperature than fresh animals, i.e., animals that had not been treated with proteins previously.

Vaughan and his collaborators^{2, 3} investigated the question of protein fever in its various phases. They showed that various types of clinical fever curves can be produced in animals by properly regulated repeated injections of foreign proteins. Vaughan⁴ points out that "the effect of protein injections on the temperature is more prompt and marked in sensitized than in fresh animals."

Pfeiffer⁵ found that animals reinjected with a sublethal dose of a foreign protein to which they had been sensitized about two weeks previously, showed a considerable fall of body temperature.

Later Friedberger and his co-workers further investigated the question of sensitization and its phenomena bearing on variations of body temperature. Friedberger and Mita⁶ showed that while animals respond with a slight rise in temperature on the subcutaneous, intraperitoneal or intravenous injection of foreign sera in suitable doses, the reaction is much more marked with infinitely smaller doses if the animal had been previously sensitized to the sera. They found, for instance, that the anaphylactic index, i.e., the ratio of the least effective dose in non-sensitized animals to the least effective dose in sensitized animals may be as high as one million, if sheep serum is injected intraperitoneally into guinea-pigs. These authors express the belief that the sensitized animal has acquired an enormously increased capacity to react owing to antibodies formed during the incubation period following the preliminary injection of the foreign protein, these antibodies now effecting a very rapid breaking up of the reintroduced foreign protein into fever producing substances, a view which had been previously expressed somewhat differently by Vaughan⁷ in explanation of the mechanism of anaphylaxis. The above named authors also point out that this reaction *in vivo* is essentially the same as that occurring *in vitro*, resulting in the formation of an anaphylatoxin which is capable of producing a rise in temperature if injected into an animal. They also showed that there is an absolute specificity of the different sera in producing the anaphylactic changes in temperature.

More recently Friedberger and his co-workers⁸ showed that the temperature phenomena of active sensitization are equally true of passive sensitization, the anaphylactic interval, i. e., the period between the injection of the immune serum and the antigen, being very much shorter in the latter.

As to the *modus operandi* of the temperature reactions in anaphylaxis there seems to be no satisfactory explanation. Friedberger who studied temperature reactions following the injection of various inorganic salts in normal animals,⁹ and in animals sensitized to foreign proteins⁸ finds that sensitization does not affect the animal in responding to the injection of salts, apparently the two phe-

*From the Pharmacological Laboratory of the University of Michigan.

nomena being of a different nature. This author suggests that in anaphylactic fever there is usually a cutaneous vasoconstriction, the blood being thereby diverted to the internal organs with consequent lessened dissipation of body heat. Whether this is the sole factor in the production of fever, or whether there is also increased heat formation by either a direct action on the heat centers, or indirectly by increased metabolism, owing to augmented movements of various smooth muscle tissues, which have been shown to occur in anaphylactic reactions, remains an open question.

In favor of the assumed decrease of heat loss which may at least partly account for the rise in temperature, speak the researches of Pearce and Eisenbray¹⁰ who studied the mechanism of anaphylactic shock produced by the reinjection of horse serum in dogs, and Edmunds¹¹ who investigated the same problem on the injection of Vaughan's "casein poison" in dogs. These authors find a fall in blood pressure due to a peripheral paralysis of the splanchnic vessels, which with the assumed constriction of the cutaneous vessels would be very effective in conserving body heat.

Hashimoto¹² injected the antigen in sensitized animals intracerebrally or directly into the "heat center" and finds that a very much smaller dose of antigen when thus applied locally is effective in producing the temperature changes than when injected intravenously. From this he concludes that the action of the antigen in producing fever is on the heat centers, the exact mechanism, however, being obscure.

This research was undertaken with a view of ascertaining if possible the more immediate causes responsible for the temperature changes observed in anaphylactic animals. We are not concerned with the remote causes. In other words, the purpose of this research is not to investigate the mechanism of anaphylaxis, but rather to learn more definitely the mechanism that causes the anaphylactic temperature changes. Is the anaphylactic rise in temperature due to increased metabolism, or is it the result of lessened heat dissipation? Is this fever comparable with neurogenic fever, or is it more in the nature of true infectious fever? What relation do such drugs as are generally held to depress the sensitiveness of the heat centers bear to this type of fever? It is questions of this character that an attempt is made to answer.

Rabbits were used through this work. A preliminary series of experiments were performed to establish a definite procedure by which febrile reactions could be invariably elicited. The method finally adopted consists in the sensitization of the animal with two cubic centimeters of beef serum injected subcutaneously, allowing an incubation period of at least two weeks, and then reinjecting the animal with one-tenth cubic centimeter of beef serum, diluted with sterile normal salt solution to one cubic centimeter, into one of the ear veins. This procedure never fails to elicit a rise in temperature of about one degree Centigrade or over within about two hours of the injection. The temperature generally returns to normal in about four to five hours subsequent to the injection. A second rise in temperature may generally be elicited in the same animal on the same day or on the following day by a repeated injection of the antigen. Smaller doses of the antigen, e.g., one-hundredth cubic centimeter injected into sensitized animals, are just as certain in producing a rise in temperature, although generally

not quite so high. Smaller doses than the last named are not certain in rabbits. If on the other hand larger doses are given, e.g., half to one cubic centimeter, the animal may actually show a subnormal temperature associated with more or less marked symptoms of anaphylactic shock, depending on the size of the dose. Two cubic centimeters of beef serum injected intravenously into sensitized rabbits results in acute anaphylactic death, according to Schürer and Strasmann.¹⁵ The temperatures were always taken per rectum, the thermometer being introduced a distance of 6 cm. It is needless to say that with each series of experiments a sufficient number of controls were carried out simultaneously.

TEMPERATURE REACTIONS IN SENSITIZED ANIMALS WITH TRANSECTED CORD.

Freund and his collaborators^{13, 14} showed that by transecting the spinal cord at different levels one could exclude the chemical or metabolic factor on the one hand, or the physical factors on the other, involved in thermo-regulation. They showed that dividing the rabbit's cord in the thoracic region results merely in a physical disturbance of heat regulation. If such an animal is subjected to a low external temperature there is more loss of heat than can be compensated for by the increased metabolism, which actually takes place under the circumstances. Such an animal does, however, maintain its body temperature within wide limits of external temperature (8° to 30° C.). If the cord is divided in the cervical region the animal becomes poikilothermic, according to these investigators. Both the chemical as well as the physical factor in thermo-regulation are disturbed and the animal is entirely dependent upon its surrounding temperature. It was thought, therefore, that the reaction of sensitized animals with transected cord to the reinjected antigen might give, if not final proof, at least a suggestion as to the nature of the anaphylactic febrile reaction.

Sensitized rabbits were lightly anesthetized with ether and under aseptic precautions had their spinal cords transected at definite levels. After the operation the animals were properly cared for, were kept warm at fairly constant room temperature (20°-22° C.), and were under observation for from 24 to 48 hours before the effects of the reinjected antigen were studied.

It may be observed on passing that the animals with severed cord below the cervical region recovered from the operation shortly and appeared quite normal thereafter, except for the paralysis of the hind legs, bladder and the sphincters.

The protocols of the experiments are detailed below.

Exp. 17. Rabbit, sensitized Dec. 6, 1915.

Dec. 29, 12 M.	Temp. 39.1	Ether. Transected cord at thoracico-lumbar region.
Dec. 30, 1 P. M.	Temp. 38.7	Injected 0.1 c.c. beef serum intravenously.
2 "	" 38.6	
4 "	" 38.3	
5 "	" 38.1	
8 "	" 37.8	
Dec. 31, 10 A. M.	" 37.8	
11 "	" 37.7	Injected 0.05 c.c. beef serum intravenously.
1 P. M.	" 37.5	
2 "	" 38.1	
4 "	" 39.1	

Exp. 20. Rabbit, sensitized Dec. 7, 1915.

Dec. 29,	2 P. M.	Temp.	39.1	Ether.	Transected cord at thoracico-lumbar region.
Dec. 30,	10 A. M.	"	38.9		
	1 P. M.	"	38.9	Injected	0.1 c.c. beef serum intravenously.
	4 "	"	37.9		
	5 "	"	37.7		
	8 "	"	38.3		
Dec. 31,	11 A. M.	"	38.3	Injected	0.2 c.c. beef serum intravenously.
	1 P. M.	"	37.1		
	4 "	"	38.3		
	6 "	"	38.3		

Exp. 22. Rabbit, sensitized Dec. 9, 1915.

Dec. 29,	3 P. M.	Temp.	39.3	Ether.	Transected cord at thoracico-lumbar region.
Dec. 30,	1 "	"	39.1	Injected	0.1 c.c. beef serum intravenously.
	4 "	"	38.6		
	5 "	"	38.4		
	8 "	"	38.4		

Exp. 24. Rabbit, sensitized Dec. 11, 1915.

Jan. 3,	4 P. M.	Temp.	39.1	Ether.	Transected cord at thoracico-lumbar region.
Jan. 4,	10 A. M.	"	38.2		
	12 M.	"	37.9	Injected	0.02 c.c. beef serum intravenously.
	2 P. M.	"	37.9	Injected	0.1 c.c. beef serum intravenously.
	4 "	"	37.0		

Exp. 25. Rabbit, sensitized Dec. 9, 1915.

Jan. 5,	2 P. M.	Temp.	39.1	Ether.	Transected cord at 6th thoracic.
Jan. 7,	10 A. M.	"	30.9		
	11 "	"	31.0		
	2 P. M.	"	30.8	Injected	0.01 c.c. beef serum intravenously.
	4 "	"	29.6		

Exp. 26. Rabbit, sensitized Dec. 11, 1915.

Jan. 3,	3 P. M.	Ether.	Transected cord at 2d lumbar.		
Jan. 4,	2 P. M.	Temp.	38.9	Injected	0.1 c.c. beef serum intravenously.
	5 "	"	39.1		
Jan. 5,	10 A. M.	"	38.9	Injected	0.05 c.c. beef serum intravenously.
	12 M.	"	39.7		
	2 P. M.	"	40.0		

Exp. 27. Rabbit, sensitized Dec. 11, 1915.

Jan. 3,	4 P. M.	Temp.	39.1	Ether.	Transected cord at 1st lumbar.
Jan. 4,	12 M.	"	38.9	Injected	0.02 c.c. beef serum intravenously.
	2 P. M.	"	39.4	Injected	0.1 c.c. beef serum intravenously.
	5 "	"	39.6		
	8 "	"	40.3		

Exp. 28. Rabbit, sensitized Dec. 11, 1915.

Jan. 5,	2 P. M.	Temp.	39.2	Ether.	Transected cord at 8th thoracic.
Jan. 7,	11 A. M.	"	27.3		
	2 P. M.	"	27.5	Injected	0.01 c.c. beef serum intravenously.
	4 "	"	27.0		
	8 "	"	27.8		

Summarizing the results of the above experiments it would seem that depriving the animal of its physical forces normally concerned in thermo-regulation, as is done by transecting the cord in the thoracic region, is sufficient to render the animal unable to develop fever under the influence of the antigen, if it is assumed that the effect of the antigen in producing a febrile reaction is in the nature of stimulation of the higher vasomotor centers. Two experiments, not

recorded, in which the cervical cord was severed showed essentially the same results as those obtained from the division of the thoracic cord, viz., absence of febrile reaction from the antigen, but a reduction of temperature. It is therefore unnecessary to assume augmented metabolism to be the cause of anaphylactic fever, although it may act as a contributory factor. Incidentally, the results obtained here are in accordance with the work of Nebelthau, who was unable to produce infectious fever in rabbits whose cords were divided in the cervical region.

The fall in temperature in some of the cited experiments must be regarded in the same light as the reduction in temperature following the reinjection of relatively large quantities of the antigen in normal sensitized animals. It must be looked upon as a manifestation of collapse, resulting probably from anaphylactic shock of greater or less severity. Why animals with divided cords should be so much more susceptible to the antigen than normal animals is not clear, and must be the subject of further investigation.

EFFECT OF MORPHINE ON ANAPHYLACTIC FEVER.

It is generally held that morphine in small doses lessens the sensitiveness of the heat centers. Stern¹⁶ found that tetrahydro- β -naphthylamin is ineffective in producing fever after morphine. Hashimoto¹⁷ has recently come to a similar conclusion by an even more direct method. He found that morphine given in doses of 9 to 14 mgm. per kilo. subcutaneously to rabbits with "heat puncture" fever lowers the temperature of the animal, and heating or cooling of the centers is without effect. It was thought interesting to see what effect morphine would have on anaphylactic fever. A number of rabbits were sensitized on January 19 with two cubic centimeters of beef serum subcutaneously. During February 8 and 9 these animals were tested with the antigen injected intravenously. Morphine sulphate was injected in doses of from 3 to 5 mgm. per kilo. subcutaneously about 15 minutes previously to the reinjection of the antigen, to allow sufficient time for the drug to depress the heat centers. In every case was there a definite fall in temperature, as it appears from the table, developing to a maximum within about two hours and returning to normal within about four hours of the injection of the antigen. If after the temperature returned to normal a second injection of antigen was made without morphine, a typical rise in temperature followed, exactly the same as in the controls. If however a second injection of antigen was preceded by one of morphine, a fall in temperature again occurred. No symptoms whatever could be detected from the morphine, and normal animals treated with like doses of the drug showed no effects whatever on their body temperature.

TABLE SHOWING THE EFFECT OF MORPHINE SULPHATE ON ANAPHYLACTIC FEVER.

Exp. No.	Morphine sulph. mgm. per kilo.	Beef serum in c.c.	Temperature changes.
30	5	0.1	-1.0
31	5	0.1	-0.6
32	3	0.1	-0.5
33	3	0.1	-0.5
34	3	0.01	-0.6
35	3	0.01	-0.6
37	5	0.01	-0.2

It is evident therefore that morphine not only completely prevents the anaphylactic rise in temperature, but actually causes the reinjected antigen to lessen body temperature in sensitized animals, and produces a condition analogous to that obtained by separating the higher centers from the spinal cord.

It is noteworthy that the failure to obtain a rise in temperature from the reinjection of the antigen after morphine is parallel to the findings of Mendelson¹⁸ who was unable to produce fever in dogs through pus injections when the animals were under the influence of morphine.

GLYCOGEN CONTENT AND ANAPHYLACTIC FEVER.

It is a well recognized fact that experimental fever may be induced in animals by the "heat puncture" operation of Aronsohn and Sachs,¹⁹ whereby the corpora striata are injured. This type of experimental fever has been the subject of a great deal of investigation. Rolly²⁰ studied the problem of "heat puncture" fever with relation to the glycogen content of the liver and muscles, and he was able to show that the temperature of animals whose livers were freed from glycogen were unaffected by the operation of "heat puncture." He furthermore showed that infectious fever could be induced in animals irrespective of their glycogen content. There is thus a vast difference between infectious fever and "heat puncture" fever, or as it is termed neurogenic fever. Lusk²¹ states: "The rise in temperature after puncture of the corpora striata . . . is like the glycosuria following Claude Bernard's puncture, in that its mechanism is no more invoked in true infectious fever than are the nerve centers in diabetes mellitus."

The following experiments were undertaken to see what relationship there is between the febrile reactions in anaphylactic animals and their glycogen content. Rabbits were sensitized in the usual manner, and after a sufficiently long incubation period they were made to fast for several days, and were subjected to strychnine convulsions for about two hours. After complete recovery, the animals were tested with the antigen to see whether they could still develop fever or not. At the completion of the test the animals were killed by a blow on the head, the liver and the muscles of the hind leg were immediately removed, weighed, and their glycogen content quantitatively determined according to the method described by Pflüger.²²

The method is briefly as follows: The tissues after having been removed from the animal and weighed are transferred into a casserol containing boiling 1.7% KOH. This is kept boiling for half an hour, or until the tissues are thoroughly digested. The whole amount or an aliquot portion thereof is neutralized with HCl, and HgI₂ in dilute HCl is added as long as any precipitate continues to form. This is now filtered and washed. To the filtrate two volumes of 96% alcohol are added, and the glycogen is allowed to separate out over night. The precipitate should be redissolved in 15% KHO, reprecipitated with HgI₂ in HCl, filtered and washed, and the filtrate treated with alcohol, as above. Generally this is quite sufficient, but if there is any reason to suppose that not all the glycogen was washed out, the same procedure may be repeated a third time. On the following day all the filtrates containing the glycogen are filtered, the glycogen transferred to the filter paper, washed with alcohol, then with ether, and dried at 98°-100° C. to a constant weight.

Exp. 38. Rabbit, 1.82 kilo. Sensitized Jan. 19.

Feb. 7, fasting begun.

Feb. 9, convulsions induced by the repeated injections of strychnine sulph. in 0.2 mgm. doses given subcutaneously every 10 min. Convulsions were kept up about two hours. Fasting continued.

Feb. 12, 9 A. M. Temp. **38.8** Injected 0.1 c.c. beef serum intravenously.

10 " " 39.3

11 " " 40.1

2 P. M. " 39.3

The animal was then killed, liver weighed 35 gm., muscles removed weighed 60 gm.

Feb. 13, glycogen determined:

1. Muscles 0.03%

2. Liver 0.45%

Exp. 39. Rabbit, 1.52 kilo. Sensitized Jan. 19.

Feb. 7, fasting begun.

Feb. 10, strychnine convulsions lasting about two hours.

Feb. 11, 10 A. M. Temp. **38.8** Injected 0.2 c.c. beef serum intravenously.

11 " " 38.8

2 P. M. " 39.9

Animal killed. Liver weighed 32 gm., 40 gm. of muscles removed.

Feb. 12, glycogen determined:

1. Muscles Trace

2. Liver 0.075%

Exp. 42. Rabbit, 1.92 kilo. Sensitized Jan. 19.

Feb. 8, fasting begun.

Feb. 12, strychnine convulsions lasting about two hours.

Feb. 16, 9 A. M. Temp. **38.7** Injected 0.1 c.c. beef serum intravenously.

11 " " 39.3

1 P. M. " 39.6

3 " " 39.0

Animal was killed. Liver weighed 36 gm. Muscles removed weighed 60 gm.

Feb. 17, glycogen determined:

1. Muscles 0.03%

2. Liver 0.2%

A survey of the outlined experiments shows that the rise in temperature in sensitized animals following the injection of the antigen is independent of the glycogen metabolism, for the promptness of the febrile reaction and the height it reached in these animals in which the glycogen content was reduced to a minimum amount compared with those of normal animals with normal glycogen content. It was not possible, however, in these experiments to reduce the glycogen to zero, probably because during the period of rest following the strychnine convulsions small amounts of glycogen such as have been found might have been formed from the animal's own proteins which it metabolized. A different method was therefore resorted to in the hope of eliminating all traces of glycogen.

Sansum and Woodyatt²³ have shown that fasting dogs are most effectively deglycogenized by phlorizinization and subcutaneous administration of epinephrin at three hour intervals until the D:N ratio reaches a constant figure, or until the glycosuria is no more affected. This method was applied to rabbits that had been sensitized previously to beef serum. Upon the completion of the experiments the animals were killed, and the liver and muscles were examined for glycogen.

Exp. 47. Rabbit. Sensitized June 26.

July 10, fasting begun.

11, phlorizin and epinephrin treatment begun.

15, 7 A. M. Temp. 38.8 Injected 0.1 c.c. beef serum intravenously.

9 " " 40.0

11 " " 39.6

The animal was then killed. No glycogen was found in the muscles, and a mere trace in the liver.

Exp. 48. Rabbit. Sensitized June 26.

July 10, fasting begun.

July 14, phlorizin and epinephrin treatment begun.

17, 10 A. M. Temp. 39.2 Injected 0.1 c.c. beef serum intravenously.

12 M. " 39.9

2 P. M. " 39.2

No glycogen was found in either muscles or liver.

Exp. 51. Rabbit. Sensitized June 29.

July 17, fasting begun.

19, phlorizinization begun.

21, 10 A. M. Temp. 39.3 Injected 0.2 c.c. beef serum intravenously.

12 M. " 40.1

3 P. M. " 40.3

4 " " 39.4

No glycogen was found in the muscles. The liver weighed 33 grams, and contained 0.1% glycogen.

Exp. 52. Rabbit. Sensitized June 29.

July 17, fasting begun.

19, phlorizinization begun.

21, 10 A. M. Temp. 38.8 Injected 0.2 c.c. beef serum intravenously.

12 M. " 40.3

1 P. M. " 40.7

3 " " 40.5

No glycogen was found in the muscles. The liver weighing 38 grams contained 0.04% glycogen.

Exp. 53. Rabbit. Sensitized June 29.

July 17, fasting begun.

21, phlorizinization begun.

24, 8 A. M. Temp. 39.1 Injected 0.2 c.c. beef serum intravenously.

10 " " 39.5

12 M. " 40.1

2 P. M. " 39.9 No glycogen was found in the muscles. The liver weighed 40 grams, and contained 0.17% glycogen.

Exp. 54. Rabbit. Sensitized June 29.

July 17, fasting begun.

21, phlorizinization begun.

24, 8 A. M. Temp. 39.1 Injected 0.2 c.c. beef serum intravenously.

11 " " 39.9

2 P. M. " 39.1 No glycogen was found in the muscles, and but a mere trace in the liver.

These experiments show quite conclusively that the anaphylactic rise in temperature has nothing whatever to do with the glycogen metabolism, and that it is of an entirely different nature from neurogenic fever.

The relation of anaphylactic fever to the glycogen content of the animal,

its relation to morphine, a drug that depresses the sensitiveness of the heat centers, and finally its relation to the transection of the spinal cord, as pointed out earlier in the paper, make it at least highly probable that it is of the same nature as true infectious fever.

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THE BEHAVIOR OF CERTAIN NITROGENOUS METABOLITES IN THE BLOOD IN NEPHRITIS*

BY HOWARD T. KARSNER, M.D., CLEVELAND, OHIO.

ALTHOUGH it has been known since 1836 that Bright's disease is accompanied by an accumulation of urea in the blood yet it is only within the past few years that the subject has been studied intensively, the stimulus having been furnished by the development of methods for the determination of various nitrogenous bodies in small amounts of blood. Thanks for this service are due especially to Folin, Denis, Benedict, Marshall and Van Slyke. The purpose of this article is to summarize the numerous studies which have appeared on this general subject from clinics and laboratories more particularly in this country. Following a consideration of the results of certain studies on experimental animals, the clinical work will be reviewed and correlated as far as possible with the symptomatology of Bright's disease. The particular metabolites dealt with in this review are the total non-protein nitrogen, urea, creatinin and uric acid. The reason for omission of a consideration of the ammonia, amino-acids and undetermined nitrogen is that these substances, owing to difficulty in the methods, have not received very full study.

*From the Department of Pathology of the Medical School of Western Reserve University.

In experimental studies two methods of attack have been employed, operative reduction of the amount of renal tissue and the use of various poisons or nephrotoxic agents to produce different forms of nephritis. Removal of one kidney from an animal leads to a slight accumulation of nitrogenous metabolites in the blood, which lasts for less than twenty-four hours. Removal of one kidney and half its fellow (three-fourths of the entire kidney substance) leads to a moderate accumulation which disappears in less than three days. Removal of both kidneys leads to a progressive accumulation which when plotted out yields a practically straight line of increase, the animal dying in three to four days. How much of this accumulation is due to the fault in excretion and how much is due to altered metabolism, particularly increased protein catabolism, is not known. That the latter process is of some importance in nephritis can hardly be doubted.

The nephrotoxic agent which has received the most careful study is uranium, used either as the acetate or nitrate. Studies of dog, cat and rabbit have repeatedly shown very material accumulation of total non-protein nitrogen and of the urea nitrogen. Other forms of experimental acute nephritis studied less extensively are those produced by chromate, cantharadin, diphtheria toxin, specific hemolytic immune serum, arsenic and tartaric acid. Such experimental studies are difficult of interpretation because of the impossibility of producing a pure glomerular or pure tubular nephritis, as well as the fact that other organs are simultaneously attacked by the poisons. The opinion of the writer is that although undoubtedly the forms showing predominant involvement of the glomeruli may lead to very considerable accumulations of nitrogenous metabolites, yet on the whole the anatomical involvement of the tubular epithelium is distinctly of more significance from the point of view of functional disturbance. If such studies as these have failed to reveal much of fundamental importance yet they have served to blaze the way for the utilization of essentially the same methods for the study of human nephritis.

At the outset of the consideration of human nephritis it must be borne in mind that most of the studies have been made on cases, the diagnoses of which have not been controlled by post-mortem study of the kidneys. This is a fault which can be corrected only by many years of detailed study of cases both clinically and pathologically and will be much aided by co-operative simplification of classification by pathologists and clinicians. Chemical studies have been almost entirely confined to chronic nephritis of the type commonly called interstitial, rather than the acute forms of the disease. With the best technique it is difficult to believe that there is much possibility of very intimate correlation between morphological lesion and functional disturbance, especially in view of the fact that carefully controlled experimental work has failed to yield much information. Although from the point of view of evolution and embryology the kidney is a duplex organ made of glomeruli and tubules yet the human kidney appears to operate functionally as a unit, disturbance of one constituent of which is invariably accompanied by greater or less disturbance of the remaining constituents.

It can safely be said that the presence of Bright's disease leads sooner or later to an accumulation of the total non-protein nitrogen of the blood and that as

a rule the urea fraction shares in the accumulation. Indeed the latter usually shows a considerably greater percentage increase than the former. It must be remembered, however, that this rule is not without exception and cases of nephritis may be well recognizable by ordinary clinical study and fail to show increase of non-protein nitrogen of the blood. Factors of considerable importance in the estimation are the nitrogen and water intake, for it has been shown that increase or decrease of nitrogen in the diet will similarly affect the blood of nephritics and in the same way "dehydration" will increase and marked "hydration" decrease the relative amount of nitrogenous metabolites in the blood.

Creatinin, although readily excreted by the kidney under normal conditions, may show considerable increase in the blood of nephritics although with not such great constancy as the total nitrogen or the urea. This fact, however, would impress one with the probable prognostic significance of a distinct increase of this easily soluble and readily excreted body. On the other hand the relative insolubility of uric acid and its corresponding difficulty of excretion are reflected according to the work of V. C. Myers in an accumulation of this body early in the course of certain types of nephritis. In fact it is stated that "as the permeability of the kidney is lowered, in the type of cases studied, it becomes apparent first by a retention of uric acid, later by that of urea, and lastly, by creatinin, producing a 'staircase effect.'"

The accumulation of nitrogenous metabolites is not confined to the blood, for the other tissues of the body with the exception of the urinary tract show a marked uniformity of distribution of urea, creatinin and uric acid.

The striking manifestations of chronic nephritis, in addition to disturbances of water output, albuminuria and cylindruria, are edema, hypertension, retinitis, acidosis and uremia. Albuminuria and cylindruria are probably dependent upon factors other than general metabolic disturbance and are not to be correlated with accumulation of nitrogenous metabolites in the blood. Water excretion, however, varies very much and in those cases where so-called fixation of urinary specific gravity appears, the amount of non-protein nitrogen per 100 c.c. of blood may vary considerably with the water intake. On the other hand a large intake and output of water tends to reduce accumulations of non-protein nitrogen in the blood, particularly where this is accompanied by a low protein diet. In so far as blood pressure is concerned it is well known that although hypertension is frequently accompanied by accumulations of non-protein nitrogen this is not always the case. In fact a case which shows such accumulation may, by regulation of the diet, exhibit a normal blood content of non-protein nitrogen without altering the level of blood pressure. Conversely a high protein diet is not necessarily followed by an increase in blood pressure.

Few studies have been directed toward the relation between albuminuric retinitis and non-protein nitrogen of the blood. What data have been accumulated point to an absence of any such relation.

Acidosis appears in the course of nephritis but is an almost constant concomitant of uremia. Hence, while in the earlier stages of nephritis, acidosis is unusual and nitrogen accumulation in some form or other fairly common, when uremia appears the acidosis is almost constant and occasional cases appear in which accumulation of nitrogen cannot be demonstrated. It can easily be seen

that there is no parallelism between the degree of acidosis and of non-protein nitrogen accumulation in any stage of nephritis except in uremia and even in that condition wide variations may be found.

In connection with non-protein nitrogen accumulations the subject of edema can be dismissed quickly as having no especial bearing, being related rather to the regulation of salt balance in the body than directly to nitrogenous metabolism.

For years there have been many tests applied to indicate the functional capacity of the kidney but at the present time the phthalein test of Rowntree and Geraghty seems to be the most satisfactory of the simpler methods. Examinations of the relation of this test to non-protein nitrogen accumulation have been numerous on the basis of both clinical and experimental material. Although clinically exceptional cases are occasionally reported, it is the general experience that a diminution of phthalein output is accompanied by an increase of non-protein nitrogen in the blood and that these alterations are inversely proportional. This rule however is distinctly more constant in experimental animals than in human cases. As to other tests such as the iodide, lactose, water and chloride tests, it may be said that the iodide and phthalein tests practically parallel each other, the lactose test is still of uncertain value, the chloride test has no direct bearing on the nitrogenous metabolism, and the water test when positive depends upon insufficient excretion of solids in the urine and as has been mentioned earlier is almost certain to be accompanied by accumulation of non-protein nitrogen in the blood. It also goes without saying that an inability to excrete added urea or creatinin would be followed by at least a temporary accumulation of these bodies in the blood.

That increases of nitrogenous metabolites in the blood are not to be laid necessarily to faults of excretion has been pointed out by several workers. Mosen-thal says:—"In the interpretation of an increased non-protein nitrogen of the blood in nephritis four factors are to be considered—(1) retention of nitrogen by an insufficient kidney; (2) inspissation of the blood due to loss of water; (3) increase of protein catabolism; (4) the chemical combination in which non-protein nitrogen exists in the blood." There can be little doubt however that the first of these factors is the most important, at least quantitatively, yet the other factors, particularly the third, must not be overlooked in a rational consideration of the subject. This view expresses a general sense of dissatisfaction that has followed the widespread use of blood examinations in nephritis and is the natural reaction against a tendency to lay too much stress in diagnosis and prognosis on the results of blood analysis. In the same light may be considered the work of Ambard and his associates and the subsequent studies of McLean and Selling and of McLean. On the basis of the hypobromite method for the determination of urea in the blood and in the urine Ambard and Weill and certain other French investigators found that the rate of excretion of urea follows such definite laws that a very precise formula gave as its resultant figure a constant or coefficient. McLean, using the more accurate methods devised by Folin and others, has confirmed Ambard's observations and shows that although the concentration of total non-protein nitrogen and of urea in normal human blood is not constant but varies within wide limits according to diet, fluid ingested, etc., yet Ambard's coefficient, when computed on the basis of the methods indicated above varies in

normal individuals only between comparatively narrow limits and may be regarded as constant. McLean has applied the method with the same general results in regard to chlorides and by the introduction of a slide rule has simplified the calculations. Certain workers, however, have not met with such good results as has McLean, and Addis and Wanatabe have published a serious criticism of Ambard's laws of urea excretion. This attacks the accuracy of the fundamental observations and further publications from these writers will be awaited with great interest. In the meantime it would appear that the McLean index (Ambard coefficient regarded as unity) serves as a somewhat more accurate method of expressing the ability or inability of the kidney to excrete urea and chlorides than does the estimation of these products in the blood only.

In contrast to failure of the kidney to excrete nitrogenous metabolites it must be recognized that in certain stages, presumably early stages, of human nephritis and in the earlier stages of experimental nephritis, notably that produced by uranium, a condition of excessive excretion of nitrogenous metabolites or "superpermeability" may appear. In this condition the total non-protein nitrogen of the blood may be distinctly reduced. Comment on this phenomenon is necessarily brief because the number of cases studied is small.

In summary it may be said that accumulation of nitrogenous metabolites in the blood is a common condition in nephritis and that this accumulation is shared by the various metabolites in somewhat different degree; that whereas in certain cases those metabolites which are excreted with difficulty show earlier accumulation and those secreted with ease show later accumulation, yet the well marked cases show considerably greater accumulations of urea than of any other part of the non-protein nitrogenous material. The accumulation is in a general way in keeping with other modes of estimating renal sufficiency and with the general condition of the patient, and usually increases as uremia develops. Early cases may show normal blood and occasional cases of superpermeability may show decreases in the total non-protein nitrogen. There is, however, nothing infallible either diagnostically or prognostically in the chemical examination of the blood, for wide variations of all kinds may be found. On the other hand a consideration of all the features of a case, its general condition, its water and food intake, its excretions other than urine, the progress of the case clinically, the information yielded by other clinical and chemical tests, when added to the results of blood examinations furnish the basis for a balanced judgment of the case and serve to make the diagnosis accurate, the prognosis correct and the treatment rational.

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LABORATORY METHODS

A NEW CONTAINER FOR PATHOLOGICAL SPECIMENS*

BY CARL KELLNER, NEW HAVEN, CONN.

OWING to the fact that it is difficult to obtain an oil-tight closure in the ordinary glass museum jars for the preservation of specimens prepared by the Pick-Kaiserling method, I have found an excellent substitute for them which possesses several distinct advantages. The new containers, made of sheet celluloid, are very light, and can be constructed in any desired size: They are adapted particularly for small and medium size specimens. Very large specimens, on account of the weight, do not lend themselves so well to permanent



Fig. 1.

preservation in these containers. The latter, moreover, are cheaper and do not break. An idea of the form of these containers can be obtained from the accompanying photographs. Figs. 1 and 2 not only show the form of the celluloid preserving jar, but also the clarity with which the specimens can be demonstrated.

The containers are very simple to make and for this purpose clear sheet celluloid one-thirty-second of an inch thick is used for the front and back, and a similar material one-sixteenth of an inch thick for the sides. They are manufactured as follows: The sheet celluloid is cut in the required sizes by means

*From the Surgical Laboratory, Yale University.

of a photographic print cutter. The front and one adjacent side piece are sealed with a ten per cent solution of celluloid in acetone and pressed together. The same process is repeated on the opposite side. The back is then sealed to the two side pieces in a similar manner. The bottom and top segments are cut to fit the inside measurements of the container. The inner edge of the box receives a coating of the binding solution and the bottom piece is pushed into the end. All of the joints are now protected by an extra coating of the celluloid



Fig. 2.

solution and the container is ready for the specimen. I have found it convenient often to make frames or supports of small strips of celluloid to hold the specimen suspended in the solution to which it may be attached by means of a silk thread. The strips may be fixed to the side by a small amount of the celluloid solution, thus fixing the specimen in any desired position. The container is now filled with the Russian oil preserving fluid and the top pushed in, after the upper edge has been moistened with the celluloid solution. Should there be any

leaks, it is only necessary to wipe away the oil and put on a small amount of the binding solution, in order to render it oil tight. The sides of the container are now painted with a quick drying Jap-a-lac and the typewritten label attached on the front.

While there is a faint yellowish tinge to the celluloid, the specimens are seen more distinctly than in the glass jars. This fact can be seen by a study of the photographs. Specimens mounted this way have been dropped on the floor without breaking. They likewise can be passed around for the inspection of a class with much greater ease and safety than the ordinary museum jars. It has been thought that other laboratories might find this method of preservation of pathological material for demonstration purposes more convenient than the glass jars, as we have done.

A SIMPLE METHOD OF CONCENTRATING TUBERCLE BACILLI IN SPUTUM AND URINE

BY WILLIAM KRAUSS, M.D., AND J. S. FLEMING, M.D.,
MEMPHIS, TENN.

FOR SPUTUM.

PLACE 5 c.c. sputum in a 15 c.c. centrifuge tube, add 5 c.c. of a 10 per cent sodium chloride solution and shake in a shaking machine, or by hand if none available, until it is a thin homogenous fluid as free from clumps as possible. Add 0.5 c.c. of gasoline and repeat shaking process until the gasoline is thoroughly emulsified. Centrifuge at a low rate of speed until the gasoline forms a supernatant liquid immediately beneath which is a scummy layer in which tubercle bacilli will be found, if present in original specimen.

FOR URINE.

Place 10 c.c. urine in 15 c.c. centrifuge tube and centrifuge three minutes at a rapid rate of speed. Pour off supernatant fluid. To the sediment add 10 c.c. of original urine and 1 gram of sodium chloride. Shake thoroughly or until the sodium chloride is dissolved, and add 0.5 c.c. of gasoline. Shake again for about five minutes, then centrifuge slowly. The scum beneath the gasoline contains tubercle bacilli, if present.

The use of gasoline embodies the same principle as would the use of ligroin. It gives good results, is cheaper and has proved much more satisfactory to us than the antiformin-chloroform method.

Often instead of using a centrifuge tube, a large size, narrow-neck Babcock milk bottle is preferable, in that it affords greater concentration of the scum containing tubercle bacilli.

A trace of egg albumen to slide surface before making smear secures better fixation.

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EDITORIALS

Pharmacological Reactions and Lesions in Nerve Tissues

A RECENT paper by Kocher^{1, 2} has taken up anew an old problem upon which a great amount of work, and a very much greater amount of speculation, has been expended in the past. Kocher's work deals with the histological changes produced in nerve cells by fatigue varying in degrees up to complete exhaustion. A series of fifteen complete experiments were performed, the animals used being dogs, cats, pigeons, sparrows, frogs and rats. Every experiment was carefully controlled by a resting animal of the same species, of the same approximate age and size, and the material from the two was given identical treatment, except for the activity. The nerve cells studied were from the cruciate gyrus, from the cerebellum and the anterior horn of the spinal cord, and from the dorsal ganglia. In one of the experiments over 3,500 nerve cells, classified into thirteen types according to the histologic characters, were counted to determine the relative frequency of characteristics which might be correlated with grades of activity. One feature of Kocher's investigation involved the making of a large series of camera lucida drawings under carefully controlled conditions. These drawings were used to measure and compute the area of over a thousand cells and nuclei by means of the polar planimeter. This work was carried out

apparently with great care and with full and deliberate consideration of the work of previous investigators in this field, including Hodge³, Nissl^{4, 5}, Mann⁶, Dole⁷, Lugaro⁸, Crile⁹, Verworn¹⁰, etc. Certain obscuring features involved in carrying out a piece of work of this kind are specially emphasized by Kocher. These include, (1) the difficulty of separating the effects of normal activity from unavoidable shock or injury to the nervous system in killing the animal (*the nervous system does not "die" as soon as the heart stops beating*): (2) postmortem changes ensuing between the time of death and complete penetration of the tissue by the fixing agent, owing to the action of autolytic enzymes present in all tissue; (3) varying chemical action of fixing agents; for example, formaldehyde coagulates protein by combination with the amino acid groups, alcohol by dehydration, sublimate by formation of salts, etc.; (4) the solvent action of materials used in fixation and in embedding; for example, alcohol, xylene, paraffin; (5) varying effects of chemical reaction between basic or acid dyes used in staining and the different cell structures, and (6) effect of subjecting tissue to temperature of from 50° to 54° C. (122 to 129.2 F.) in the paraffin oven for a period of several hours. Kocher's work leads him to draw the following important conclusions: There could be found no constant difference in the size of the nerve cells or nuclei resulting from activity. An apparent difference in size which appeared here and there on counting a small number of cells was shown on enlarging the series to be counterbalanced by a similar variation on the part of the controls. Hence it must be concluded that any difference in the size of cells found was within the limits of normal variation. Furthermore, in no experiment did the histologic structure of the nerve cell following activity, even to the point of exhaustion, show any constant deviation from that of the corresponding resting cells of the controls.

The striking contrast between these observations and the views on which the great bulk of the present day literature along these lines has been built up must command immediate attention. For the problems involved here are much wider than those which concern only fatigue, shock, etc., in the ordinary significance of those terms. From a strictly physiological standpoint one would, of course, scarcely consider fatigue, exhaustion, etc., from the standpoint of the central nervous system alone. For, perhaps, in all forms of activity in which changes in the central nervous system or dorsal ganglia sufficient to be detected histologically might be produced, there will also be involved extensive action of peripheral nervous structures. And these outlying structures are entitled to due consideration. Kocher's work is of special interest in this connection. For, if according to these newer observations, structural changes cannot be detected in the motor cortex or anterior horn cells, then is it possible to observe any histological variation in such structures as the sympathetic ganglia or the endings of motor nerves in striated muscle? Experimentally it is not rare for an animal in shock to manifest symptoms and reactions quite similar indeed to those presented by another animal in which the brain has been destroyed or from which the head may have been entirely removed.

There are a large number of drugs whose actions are concerned with the nervous system, either centrally or peripherally, or both. Among these may be mentioned the alcoholic beverages, opium and its derivations, cocaine and its

allies, absinthe, cannabis indica, the hypnotics, etc., whose actions are usually mainly central under ordinary conditions. Caffeine, perhaps atrophine, strychnine, picrotoxine, etc., might be placed in a slightly different category, and alcohol, lead, arsenic, phosphorus, mercury, etc., often show an especial affinity for the peripheral nerves. The drugs which act on peripheral nerve endings (myoneural junctions, receptors, receptive substance, etc.) are very numerous. In fact there is scarcely a single element in the whole nervous system which may not be acted on in a fairly direct manner by some drug. Possibly this is true also in a general way for the toxins produced in various infectious diseases. This field is, however, unfortunately very obscure. The element of time must be duly considered in the production of many of these drug reactions, for example, an alcoholic neuritis or a lead wrist drop may not be produced in a few minutes.

While the conditions involved in drug reactions are, perhaps, of a somewhat different nature from those concerned in Kocher's experiments, yet a great deal of work has been done along similar lines by the use of drugs, especially so far as concerns the action of alcohol on the cells of the central nervous system. And presumably, for corresponding periods of time during which fatigue exercises or the action of the alcohol continued, we would be inclined to expect somewhat comparable results to be produced in the histological appearance of the cells of the brain and cord. This, of course, does not necessarily follow, but in view of these recent observations it would be desirable for more work along these lines to be carried on with the use of drugs.

Experimentally it is often easily possible to demonstrate very extensive changes produced in nervous structures even by exceedingly small amounts of some substances. The methods for demonstrating these changes, however, have not generally been along histological lines. But perhaps this is only a relative matter for such a lesion as a curare paralysis¹¹ of a motor nerve ending while easily demonstrable in a few moments by electrical stimulation of the nerve trunk might after a sufficient lapse of time during which the drug was allowed to act continuously be very well demonstrated histologically. A peculiar interest attaches at present to observations along these lines for the very large number of patients with nerve lesions, both central and peripheral, which must now and for a long time to come occupy the attention of many physicians, is a matter of the greatest concern. And since much of the older work regarding the electrical diagnosis and treatment of nerve lesions, especially peripheral paralyses, is now rapidly being shown to be useless or worse, new observations are more than welcome. The older ideas implied by such terms as "faradic response" and "reaction of degeneration" are now recognized to be based on erroneous observations and even the terms themselves should be dropped from the literature¹². For the electrical response of a muscle whose motor nerve has been severed is a relative matter depending quite as much on the character, duration and frequency of the electrical impulses used in stimulating as it does on the character and extent of the degeneration which the nervous elements may have reached. Langley and Kato¹³ have recently made interesting observations along these lines, noting among other things that muscles from about the fourth day after section of their nerves are in a state of continuous fibrillation—i.e., the separate muscle fibers contract rhythmically, but with different rhythms; the muscles present a

shimmering appearance when viewed by light reflected from their surface. The contractions cause no movement of the muscle as a whole and are easily overlooked; possibly they could be shown in man by the use of the string galvanometer. As each fiber contracts many times a minute the total expenditure of energy in the day must be considerable, and Langley¹⁴ concludes that it is "reasonable to suppose that this continued activity of the muscle fibers must cause fatigue, and that the atrophy of muscle may be due to too great rather than too little functional activity." Whether or not it is possible to produce reactions corresponding to these by the use of paralyzing drugs, or to detect such lesions in the early stages by histological methods remains for future investigation.

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—D. E. J.

The Nerve Control of the Thyroids and its Relationship to Adrenal Activity

BY clinical and pathological studies it is often possible to show that disease of one organ is accompanied or followed by secondary changes in another organ, thus indicating that some intimate association may exist between the two in the normal functioning of the organism. Sometimes, however, claims that such association exists are supported only by evidence of a very circumstantial nature, as has been the case in the so-called polyglandular hypothesis concerning the cause of diabetes, according to which it is supposed that the pancreas, the adrenals, the thyroids, and the parathyroids have a special mutual relationship in connection with the utilization of carbohydrates in the animal body. Such speculative hypotheses, although they very frequently fall to pieces when thoroughly put to the test by other investigators, often serve to arouse interest in new possibilities and to stimulate investigation along new lines. Recently several important contributions having a very direct bearing on the conditions of activity in the endocrine organs or ductless glands have been made by Cannon and McKeen Cattell¹ and by Stewart, Rogoff, and Gibson.²

Cannon and Cattell have utilized the current of action which accompanies glandular activity to show that the thyroid gland becomes active: (1) when its sympathetic, but not its cerebral nerve supply is stimulated; (2) when adrenin is present in excess in the blood; and (3) when the adrenal gland is rendered

hyperactive by stimulation of its nerve supply through the great splanchnic nerves. This action current was detected by connecting the gland and the neighboring connective tissue or the trachea, to a compensated galvanometer by means of suitable unpolarizable electrodes. That such a current is a reliable indication of secretory activity was demonstrated by comparing the secretion of a typical secreting gland (i.e., one with an external secretion), namely, the submaxillary, with the occurrence of the action current. In confirmation of previous work it was a very easy matter in this case to show that secretion produced by stimulation of the cerebral and sympathetic nerves of the gland was accompanied by a definite electrical change. But this fact, taken alone, might depend, not on the activity of the gland cells themselves, but on changes in blood supply or contraction of arterial muscle or movement of fluid along the ducts; all of which changes accompany the activity. That the action current is a manifestation solely of the secretory process was shown by the following facts: (1) the current was still set up when the blood supply of the gland was cut off or the flow of secretion through the ducts was stopped; (2) it was absent when there was no secretion, but each of the other conditions attendant on secretion was induced.

The conclusion that the thyroids are innervated through their sympathetic nerve supply does not however depend alone on the evidence supplied by the observations of Cannon and Cattell on the action current. Evidence has also been contributed by the discoveries of morphologists of nerve fibers proceeding to the thyroid from the cervical sympathetic ganglia, and of the existence of non-medullated fibers reaching to the cells of the thyroid glands. Furthermore, it has been observed that severance of these sympathetic nerves, but not of the nerve supply derived from the vagus, causes atrophy of the thyroid, whereas their stimulation produces a diminution in the iodine content of the gland.

The indifference of the gland to impulses conveyed to it by way of its cerebral autonomic nerve supply (vagus) was demonstrated by Canon and Cattell by finding that the action current still occurred after severance of the superior and recurrent laryngeal nerves through which such vagus fibers would run, but on the other hand it was not observed when the vagus was excited either by applying electrical stimulation to its main trunk or by injecting the alkaloid pilocarpine, which, it will be remembered, is a specific excitant of cerebral but not of sympathetic autonomic endings.

Convincing as these experiments would appear to be, some doubt has been expressed as to whether they justify the conclusion that the nerves are really secretory in function, or whether the secretory effects produced by their stimulation may not depend on curtailment of blood supply on account of the vasoconstrictor fibers also contained in the nerves. The validity of this criticism is further supported by the discovery of Watts and Carlson that anemia of one lobe of the thyroid lessens the iodine content of that lobe. Cannon and Cattell refute this evidence by showing, first, that complete anemia of the gland produced by clamping the carotids causes no thyroid action current, and, secondly, that adrenin injected in such amount as to cause, not constriction, but dilatation of blood vessels does produce the current. It may be pointed out here that the first of these experiments cannot be considered as convincing, because the anemia must have

been equally pronounced in the tissues upon which both electrodes rested (i.e., thyroid and subcutaneous tissue), so that no difference in electrical potential might develop between them. To the second point may be raised the objection that no evidence is furnished, in the particular experiments in which adrenin was used, that vasodilatation did actually occur, and even if it did, that increased, as well as decreased blood supply might not cause currents to be set up in the thyroid gland. The possibility also exists, that contraction of the muscle fibers in the arterial walls might be the cause of the current. That such vaso-constriction in the submaxillary gland caused no current does not necessarily indicate that in the thyroid this would be the case.

Of what importance the sympathetic nerve supply to the thyroids may be in the normal control of these glands, the authors do not commit themselves; but they point out that the fact, established by Manley and Marine, that thyroid tissue grows and functions and reacts as the normal gland would do, when it is transplanted to various parts of the body, does not throw doubt on the existence in the normal gland of specific secretory nerves. It is pointed out that the heart and the adrenals still continue to give service after their denervation, yet no one doubts the importance of their normal control through the nervous system. Although Manley and Marine's observations do not therefore *neccessarily* disprove the existence of specific thyroid nerves, they would seem to indicate that in the intact animal the nerve control of the thyroid cannot concern other than some prompt and probably transient activity demanded by acute, and perhaps quite unusual, conditions. The influence of the gland on the metabolic functions has not so far been shown to depend on nerve supply.

Taking the evidence as a whole, it seems to the present writer that the brilliant work of Cannon and Cattell does definitely show that some sort of physiological activity is set up in the thyroid gland when its sympathetic nerve supply is excited, but as to the importance of this method of control in the normal functioning of the gland, nothing is as yet definitely established.

But the above investigators do not leave the problem at this point, for they show up at least one of the conditions under which the thyroid may be caused to develop an action current by changes occurring in other parts of the animal, namely, by hypersecretion of the adrenal glands. The first step in this part of the research, as has already been stated, was to show that intravenous injections of weak solutions of adrenin cause the thyroid action current. It was then found that stimulation of the peripheral ends of the cut great splanchnic nerves, in which run secretory nerve fibers to the adrenal glands, had the same effect. But if the inferior vena cava just above the entrance of the adrenal veins was occluded and the blood, surcharged with adrenin, was thereby prevented from getting to the thyroid, no electrical response was observed until the pent up blood was released. Similar splanchnic stimulation in an animal from which the adrenal glands had been removed did not cause any thyroid action current. These results would therefore seem to justify the claim that an intimate functional association exists between the adrenal and the thyroid glands, but the question is not discussed as to whether this association is a specific one in the sense that it becomes operative with less adrenal activity than would secondarily excite

some at least of the numerous other physiological mechanisms upon which adrenin acts. It is pointed out, however, that the thyroid action current obtained with solutions of adrenin are considerably weaker, (1-100,000) than those required to develop similar action currents in other glands or in voluntary muscle. Concerning this last point the recent investigations of Stewart, Rogoff and Gibson have an important bearing. In this work the liberation of adrenin from the adrenal glands into the blood stream was studied by observing the dilatation of the pupil which occurs on the same side as that from which the superior cervical ganglion has been removed, whenever the blood supply to the pupil contains adrenin. This pupillary response can be elicited, and after the same latent period, either by stimulation of the splanchnic nerves, or by injection of adrenin solutions into the femoral vein, provided always that there is nothing to interfere with the free flow of blood from the abdomen to the head. Dilatation was often obtained with concentrations of adrenin (1-100,000) which were as weak as those employed by Cannon and Cattell, and there can be no doubt that an action current accompanies movement of the pupil.

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—J. J. R. M.

Basal Metabolism and Disease

THE efficiency of the animal machine is subject to the same laws as those which govern any other energy transformer, and can be tested by the same methods. In principle, these methods consist in a comparison between the total energy production, on the one hand, and the work performed by the machine, on the other. In a machine of high efficiency a great proportion of the energy goes to produce the particular form of work (e.g., as motion) for which the machine was designed, and a lesser proportion is uselessly dissipated (e.g., as heat) than in a machine of low efficiency. In the animal machine the useful forms of energy are muscular movement and heat, and in health these bear a certain proportion to each other. In certain diseased conditions, however, the efficiency breaks down so that too great a proportion of the energy is liberated as heat. This increase in heat production may not cause a rise in body temperature, provided the mechanism for getting rid of the heat (through the lungs and skin) is in perfect working order, but if such is not the case the temperature will rise and fever be the result.

Apart from the production of fever, the excessive liberation of heat in the animal body may throw a strain on various functions and lead to the development of symptoms which are often of an alarming character. This has been

shown by E. F. Du Bois to be the case particularly in exophthalmic goiter, a disease in which it has been known for some time that excessive metabolism is going on. Du Bois examined the heat output and the respiratory metabolism of eleven patients suffering from exophthalmic goiter of varying intensity. For this purpose he used the practically perfect respiration calorimeter recently constructed in the Russell Sage Institute of Pathology in New York, and in which a patient, either in bed or sitting up, can be kept without any discomfort for several hours. It was found that in severe cases of exophthalmic goiter the energy output was increased by 75 per cent or more over the normal average; in the less severe cases the increase was frequently found to be 50 per cent. In this disease energy is therefore being liberated in excessive amount as heat and, as in any other machine, the animal mechanism suffers and after a time begins to break down. In the goiter patient the sensation of unusual warmth, the hot skin, the perspiration, are in themselves evidence enough that excessive amounts of heat have to be got rid of in order to prevent hyperpyrexia. Similar skin reactions are also produced in normal individuals by increasing the metabolism, as during muscular exercise, but in hyperthyroidism the persistence with which the heat goes on being produced affects other functions, such as the heart, producing tachycardia and enlargement, the brain, causing the well-known mental symptoms, and the general nutritive conditions, leading to emaciation, etc. "It is possible," so writes Du Bois, "that if we were able to stimulate the metabolism of normal men for twenty-four hours a day over a period of months or years, we could reproduce all the symptoms of exophthalmic goiter."

With so excessive a combustion it might be imagined that the body furnaces were perhaps not burning properly; in other words, that the intermediary metabolism was not proceeding along the usual lines, but was of such a nature that some foodstuffs were more readily utilized than others. The low carbohydrate tolerance, so commonly noted in these cases, might be accounted for by such a one-sided metabolism. Though excessive, the metabolic processes were, however, found to be perfectly normal in type, for there was almost perfect correspondence between the energy-production as computed from the amount of foodstuffs actually metabolized (indirect calorimetry) and that which was directly measured in the calorimeter (direct calorimetry). "This and the absence of

abnormal respiratory quotients $\frac{[\text{CO}_2 \text{ (expired)}]}{[\text{O} \text{ (inspired)}]}$ shows that the law of the con-

servation of energy holds good in exophthalmic goiter," just as it does in health. Using heat production as an index of the effect of treatment, it was found that mental and physical rest causes as great a reduction as any other conservative form of treatment.

In cretinism, where hypo- instead of hyperthyroidism is believed to exist, the energy output per square meter of surface was found to be about 20 per cent below the adult value, but it became immediately raised on the administration of thyroid extract.

Du Bois and his collaborators have made similar studies of heat-output and the respiratory exchange in cases of pernicious anemia, cardio-renal disease, and diabetes. In severe pernicious anemia, the most noteworthy feature was an

increase in the demand for oxygen; in cardio-renal disease, contrary to the conclusions of certain other workers, no profound change in metabolism, either qualitative or quantitative, was found to exist. Three severe and three more chronic cases of diabetes were examined, particularly with regard to the effects of the "oatmeal" and "starvation" cures. No special influence of oatmeal, either with regard to its supposed beneficial action on the disease or with regard to its utilization, could be made out. Even in the severest cases it was found that the active symptoms can be eliminated by prolonged fasting, and that the power becomes reacquired "of oxidizing sugar first from their own body protein and later from the protein and carbohydrate of a carefully regulated diet." In such cases no evidence could be obtained, either from the sugar excretion or from the respiratory exchange, that sugar can be formed from fat. The basal metabolism, calculated for surface area, was not found to be definitely increased over the normal, a finding which is contrary to that of previous investigators. It is pointed out, however, that "the level of metabolism in diabetes is the resultant of a number of forces," some of which, such as the increased metabolism of protein, tend to make it rise, whilst others, such as undernutrition and muscular relaxation, tend to make it fall. Mild exercise was found to raise the respiratory quotient somewhat in a severe diabetic, thus suggesting the possibility that exercise may improve carbohydrate utilization.

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—J. J. R. M.

Gastric Ulcers

IT has been assumed that gastric ulcers are fundamentally the result of localized disturbances of nutrition in the stomach wall, which make it possible for the acid gastric juice to digest away the damaged tissue and so produce the typical terraced, somewhat crater-shaped, reactionless lesion. The most frequently encountered lesions, following this assumption, would be vascular ones, and vascular lesions, especially arteriosclerotic ones, are, other things being equal, bland, and not infectious. For instance, arteriosclerosis leading to limitation of the blood supply to a certain area may so reduce the vital resistance of the tissue that it can be attacked by the gastric ferments. If this be true, then it may also follow that sudden occlusion of the vessels by thrombi or by emboli produce similar lesions but ones more acute in progress. The main difference would be that in the one case the lesion would be produced slowly, while in the other, it would appear rapidly. In this latter case, the process is essentially infarction. Just as these spontaneous, so to speak, processes may produce ulcers, so also will similar processes caused by purely artificial means.

lead to the same results. Injections of adrenalin into the gastric vessels have been productive in this regard, and injection of formalin into arteries and veins may, under the proper conditions, cause endarteritis and endophlebitis and, later, ulcer formation. Mere stasis produced from the venous side, especially when it is complicated with hemorrhages into the mucosa, also may lead to gastric ulceration. If these lesions are the result of deficient nutrition, then it might be suspected that qualitative, as well as quantitative, variation in the blood might be effective, a suggestion that is supported by the appearance of gastric ulcers in chlorosis.

Beside these purely endogenous hematogenic features, trauma has been suspected of playing a part, though how important one has not been able to decide,¹ and also infection.²

The infectious point of view (in more senses than one) has been developed largely by Rosenow.³ The part played by trauma has recently been noted by Steinharter. Rosenow's work was done with anhemolytic streptococci which he was able to isolate from 96 per cent of a series of gastric ulcers removed from human beings at operation. Recently isolated cultures were injected intravenously in animals and in 60 per cent of these gastric ulcers were found. In a few animals which were allowed to live for a considerable time after their inoculation, chronic ulcers were found at autopsy. From these results Rosenow has reached certain conclusions which have been summarized as follows:

1. Anhemolytic streptococci can be recovered by a special technic from practically all gastric ulcers removed at operation.

2. The streptococci from this source possess a special affinity for the stomach which enables them to localize in this organ, when recently isolated cultures are injected intravenously into animals.

3. About 60 per cent of the animals inoculated in this manner develop gastric ulcers.

4. Streptococci can be recovered from these experimental ulcers and can be demonstrated histologically. They reach their locations by the blood stream and are deposited in the capillaries of the gastric mucosa.

5. These streptococci are identical with those inoculated.

6. Anhemolytic streptococci are, therefore, the cause of gastric ulcers in man, and these organisms reach the stomach by an hematogenous route.

Recently Celler and Thalhimer⁴ have reported their experiments on the production and bacteriology of gastric ulcers with the result that they are not able to convince themselves that anhemolytic streptococci have been proved to be the factor "which either initiates the ulceration or prevents healing." They are only able to say that the presence of streptococci of this type of lesion is suggestive.

Somewhat the same state of mind exists in many laboratory workers with this modification:—it seems entirely possible that gastric ulcers may be caused by anhemolytic but other organisms may be able to produce similar lesions. Moreover there is some evidence that bacteria of whatever type may lodge in the stomach wall *after* some other influence has prepared the field. Such influences may be traumatic or nutritive.

In 1913, Steinharter,⁵ working in the Pathologic Institute of the Cincinnati General Hospital, made a preliminary note on the production of gastric ulcers by intravenous injections of *B. coli*. His results were temporarily suggestive, but not conclusive. No doubt he produced erosions of the mucous membrane but not typical ulcers. Later, his attention was attracted to the staphylococci with which he has worked during the past many months. With these organisms he has been successful, not only in producing gastric ulcers at will, but also in producing practically all the other lesions observed by Rosenow after streptococci injections. In order to produce gastric ulcers he found however that certain things were necessary; one of these was that the organism must be cultivated in the stomach wall. The other was that to obtain growth in the stomach wall,—in order to make the conditions for growth in the stomach wall satisfactory for the organism,—trauma was necessary. The trauma he produced with acetic acid in such dilution that the trauma alone caused no subsequent gross lesion. If, in such a traumatically affected site, he implanted staphylococci, an ulcer appeared. Without the organisms no ulcer developed. The organisms cultivated for such lesions, and then injected into other animals, produced ulcers in a very large proportion of the cases. Future reports by Steinharter will deal with experimental appendicitis, myocarditis, arthritis, myositis and cholecystitis, caused by staphylococci.

The important thing in all this work, a point which most writers have perhaps overlooked, is that streptococci or staphylococci show no original preference for the stomach, and that therefore if they are the essential factor in the production of a gastric ulcer, that result is fortuitous. What one may do with an organism cultivated from a tissue is scientifically interesting but not of great practical importance, except in *recurrent* disease. In the case of tonsillitis it is of importance because in such a case the tendency is for organisms from one case of tonsillitis to produce other cases. But the streptococci from a tonsil, given access to the blood stream, have no tendency to produce gastric ulcers rather than any other lesion, and the qualities developed by organisms *within* the stomach wall have very little, if any, importance as regards other stomachs. Also the localization of a relative avirulent organism in an organ or tissue of the body probably depends upon "lowered resistance," whatever that may be, and in lowering of resistance trauma and abnormal nutritive conditions are essential.

Taking it all in all at the present time, the indications seem to be that the localization of bacteria beyond primary foci of infection in the body are more or less fortuitous, and that organisms can be taught to prefer a certain organ tissue of the body. In the case of the stomach, streptococci and staphylococci have been taught to prefer that organ, and, in them, to produce typical ulcers.

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²For early literature, see Neumann: Virchow's Arch., 1906, clxxxiv.

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⁴Celler and Thalhimer: Jour. Exp. Med., 1916, xxiii, 791.

⁵Steinharter: Boston Med. and Surg. Jour., July 17, 1913; also Lancet-Clinic, Jan. 24, 1914.

Edema of the Lungs

THE lungs obtain their arterial blood supply by the bronchial arteries which are branches of the thoracic aorta. The pulmonary supply is essentially venous in character and the pulmonary arteries are merely the means by which polluted blood is carried to the lungs for rejuvenation by removal of carbon dioxide, and addition of oxygen. These are facts which are not as a rule considered in studies upon edema of the lungs.

In most articles bearing upon pulmonary edema of the obstructive type, the factor of the blood pressure in the lungs is accepted as the prime one, and the most prominently accepted therapy is that such an edema appears when there is a disproportion between the work of the right and left ventricles. Welch's work was pioneer in this field.¹ He showed that by crushing the left ventricle (in rabbits) he produced a paralysis of that chamber, while the right ventricle still remained in undiminished action. If the arterial pressure was so low that the right ventricle was sufficiently strong to drive the blood through the left side of the heart, edema did not occur. When, however, the right auricle was not able to overcome the resistance, edema occurred, unless the right ventricle was too weak to produce a high pressure. Löwit produced edema of the lungs by raising the pulmonary arterial pressure, and concluded that obstructive pulmonary edema was due to resistance to exit of blood from the lungs together with increased blood flow to the lungs. Matsuoka, working on beri-beri, came to the conclusion that the pulmonary edema in that disease is due to weakening of the left ventricle associated with hypertrophy of the right ventricle, the two factors producing the combination which Löwit believed necessary. In a recent article detailing his researches on this problem Matsuoka² says "the general outcome of the work hitherto done seems to be as follows. Paralysis or weakening of the left ventricle is the principal cause, but is probably not the only factor, for it is necessary to take into account the state of the vessel walls in the lungs; and in certain cases, especially in nephritic or cachectic condition, this constitutes, in Sahli's view, the chief determining cause.

Matsuoka did his work with the Starling isolated heart-lung preparations, and concludes that obstructive edema takes place when certain limits of combined arterial pressure and venous outflow are exceeded. This corroborates Cohnheim and Welch. But most important are the other collateral facts adduced in this research. Matsuoka says that "gaseous metabolism and energy consumption of the heart are decreased in obstructive edema of the lung. Complete stoppage of the heart rapidly takes place as the maximum metabolism of the organ is, because of its decreased rate of action, insufficient to provide the necessary energy for its contractions. When in man this final danger is threatened * * * * * *the diseased lungs should be supplied with the oxygen necessary for the blood.*"

This work and all other previous work is exceedingly interesting because it calls attention to the fact that the primary factor in the causation of edema of the lungs is stasis in the pulmonary vessels. This factor however is not the essential one in the production of the edema. Edema of any tissue is the result of water absorption by the cells and intercellular substances and cannot be brought about by any reasonable pressure. It is, so far as is known now, lack of oxygen

which is the fundamental causative factor in edema of any sort, and lack of oxygen can be expressed in terms of acidosis.

Looking at edema of the lungs as an expression of pulmonary acidosis in this sense, one may say that it may arise whenever the arterial supply of the pulmonary tissue is decreased beyond a certain minimum. Such a decrease may be brought about by pressure within the lungs, as in passive congestion, or by interference with or reduction of, the bronchial arterial blood supply. The usual obstructive edema is evidently the result of venous stasis in the lungs. In this condition the pulmonary vessels are overfilled with blood and pressure is exerted upon the bronchial vessels. This makes for slowing of the true arterial stream and this means decreased oxidation, and swelling. Should the stasis persist, then less oxygen is absorbed by the blood, and because of this the heart (myocardium) suffers, and also the arterial blood in the bronchial circulation becomes more venous, and so the heart becomes less efficient as a pump, and the blood less efficient for preserving the chemical balance of the tissue. More edema follows.

So edema of the lungs is not a mechanical problem, but a physico-chemical one, even though the underlying factors are mechanical.

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²Matsuoka: Jour. Path. and Bact., 1915, xx, 53.

—P. G. W.

The Heart in Pneumonia

"IT is generally believed," Newburgh and Porter* say, "that the heart muscle is seriously injured in pneumonia and that heart failure from this source is a frequent cause of death in this infection." There being, however, some reasonable doubt on this point, they have made a series of experiments to discover how much truth belonged to the current belief. In one group of experiments they fed the normal ventricle with normal blood. In a second group, they fed a ventricle from a pneumonic animal with normal blood. In a third group they fed a normal ventricle with pneumonic blood; and in a fourth, a pneumonic ventricle was fed with pneumonic blood. In all their experiments dogs' hearts were used. The method of isolating the heart and of preparing the animals is described in full. The heart was fed through the left coronary artery. Pneumonic hearts were taken from dogs in which pneumonia had been caused by intratracheal infection with *B. pneumoniae* (Friedländer). Pneumonic blood was also obtained from these animals. In six of ten experiments in which the pneumonic heart was fed with normal blood, the dogs were allowed to die of the disease to be certain that the infection was a fatal one.

The outcome of the experiments was that it appears that the cardiac ventricle from dogs that have died of pneumonia contracts as well as the ventricle from healthy dogs, provided the pneumonic muscle is fed with normal blood. When a normal ventricle is fed with pneumonic blood, the contractions are

*Newburgh and Porter: Jour. Exp. Med., 1915, xxii, p. 123.

much impaired. If, however, the ventricle from a dog with pneumonia is fed with pneumonic blood, the contractions are almost normal in extent and may be normal in extent and may be normal in duration. In other words, the heart muscle becomes adapted to whatever change in the composition of the blood occurs in pneumonia, at least in that type of pneumonia which is the result of infection with Friedländer's bacillus. Whether the same thing is true for the pneumococcus, we do not know. It may also be true that in at least a large percentage of pneumonias, one has to deal with a septicemic process in which the infecting organisms become lodged in the myocardium and produce changes sufficient to bring about myocardial failure. So, after all, the experiments of Newburgh and Porter only involve one end of the problem and are only conclusive under the conditions with which they were surrounded.

—P. G. W.

Amyloid

AMYLOID has always been a puzzle to pathologists. It is one of the forms of abnormal hyaline material which appears in the organs of the body during the course of chronic diseases, especially those that are the results of infections. It has been found most consistently, perhaps, as an accompaniment of chronic bone infections, and on that account and also because of its chemical structure it has been supposed that it is associated as a rule with bone or cartilage destruction. Despite the fact that it has been a topic of widespread interest, and despite the time that has been expended in researches upon its production, little has been learned of the conditions upon which its appearance depends.

In 1853 Virchow first studied amyloid, which he considered a form of animal cellulose because of the blue reaction which was obtained by treating it with iodine. It is to this reaction, which starch also gives, that the term amyloid is due. Some years later Friedreich and Kekulé, and after them Kuehne and Rudneff, showed that amyloid was a protein, and later still, Oddi, and then Krawkow, demonstrated that it was a compound of protein and chondroitin sulphuric acid, and that therefore it is analogous to nucleoprotein which is a compound of protein and nucleic acid. Still, Hanssen and Mayeda have reported studies upon amyloid in which they were able to discover no chondroitin sulphuric acid.¹

Since Frisch in 1877 announced the experimental production of amyloid in the cornea, many investigators have made similar announcements, but in each case the incidence of the production has been very inconstant. In Frisch's series only 4 of 300 corneas showed amyloid (if indeed it was that substance). Czerny reported the production of amyloid after subcutaneous injections of turpentine, which caused suppuration. Krawkow used staphylococcus pyogenes aureus to produce chronic suppurations in a large series of animals, among them, rabbits, dogs, hens, doves, and frogs. In eight of twelve rabbits he produced amyloid in various organs. In dogs the results were negative even

¹For a discussion of Amyloid see Wells: Chemical Pathology, Phila. and London, 1914, 2nd Ed., p. 378.

after two or three months of suppuration. In pigeons the results were negative and the same was true of frogs. The most constant results appeared in hens. Nowak obtained more constant results than any other worker except Krawkow.

Recently Bailey² has observed the occurrence of amyloid in the organs of animals which he had used not with the object of producing amyloid changes but for another purpose. The animals (rabbits) had been injected in the ear vein with beef extract broth cultures of colon bacillus. They were treated every 2-4 days with 1 c.c. of 24-48 hour cultures, though older cultures were occasionally used. The treatment did not produce suppurative lesions in most cases. The results were constant in that amyloid was found in all rabbits, eight in number, which were injected over a period of 88 days or more.

This is the first series of experiments in which the results were completely consistent, and they are exceedingly suggestive, and although they do not tell us anything about amyloid, they point the way to be followed in completing the work. Colon bacilli have been used before in experimental amyloid work with negative results (Davidsohn), and Nowak observed amyloid in one hen injected with sterile filtrate of colon bacillus culture. Nevertheless Bailey was unable to get the same results, with other organisms, which he was with coli.

—P. G. IV.

²Bailey: Jour. Exper. Med., 1916, xxiii, 773.

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